MUSEUM NATIONAL  **D'HISTOIRE NATURELLE**

Ecole Doctorale Sciences de la Nature et de l'Homme – ED 227

Année 2011 **N°attribué par la bibliothèque**

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THESE

Pour obtenir le grade de

DOCTEUR DU MUSEUM NATIONAL D'HISTOIRE NATURELLE

Spécialité : Systématique évolutive

Présentée et soutenue publiquement par

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Le 28 juin 2011

Variabilité et dispersion au sein du genre *Dicranum* Hedw. (*Dicranaceae, Bryophyta*) : approches moléculaire et morphologique

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"*The beauty there in the mosses must be considered from the holiest, quietest nook*"
Henri David Thoreau, Natural History of Massachusetts (1842)

Remerciements

Ces trois intenses années de recherche dans l'univers de la Bryologie, n'auraient pas vu le jour sans la rencontre entre les différents protagonistes, il y a de cela un peu plus de cinq ans, à l'occasion de mon stage de master 1. Je remercie donc Jacques Bardat et Catherine Rausch de m'avoir fait confiance et de m'avoir offert la possibilité de découvrir et d'aimer, ce monde peuplé d'organismes cryptiques.

Les années passant, avec deux stages à la clé, la perspective de réaliser une thèse s'est précisée et concrétisée. Merci à Nathalie Machon, Marie-Catherine Boisselier et Pierre-Henry Gouyon d'avoir participé à la conception du sujet de thèse. Milles merci à Jacques d'avoir accepté d'être mon directeur de thèse et de m'avoir patiemment transmit son savoir de taxonomiste et de botaniste appliqué…

Ce travail de thèse n'aurait pas pu avoir lieu sans le soutien et l'aide de nombreux bryologues nationaux et internationaux : Alain Untereiner, Vincent Hugonnot, Frederic Ritz, Jean-Claude Vadam, Cao Tong, Martin Nebel. Merci à eux pour m'avoir accueilli chez eux le temps d'une mission, et de m'avoir fait découvrir leurs régions. Merci à tous ceux qui ont pris le temps de récolter et de m'envoyer des échantillons qui ont permit de réaliser cette première phylogénie moléculaire : Bernard Goffinet, Claudine Ah-Peng, Min Thuan, Jean Faubert, Lars Hedenas, Michael Ignatov et sa fille (pour le transport des spécimens), Nele Ingerpuu, Masanobu Higushi, Sanna Laaka-Lindberg, Angela Newton, Cecilia Sergio, Scott Schuette, Bruno de Reviers, Serge Müller, Torbjörn Tyler, Simon Laegaard, Jacques Bardat.

Merci aux membres de mon comité de thèse pour m'avoir suivi tout au long de ces trois années.

Merci à Alexandre Hassanin pour son aide et ses conseils toujours judicieux sur les analyses moléculaire. Je te dédicace les gabonais…

Un grand merci à Corinne Sarthou, Catherine Reeb, Robbert Gradstein et Denis Lamy pour leur patience, leur aide et leurs encouragements.

Ce travail s’est réalisé en garde partagée entre le 12 rue Buffon et le 43 rue Cuvier. Je remercie donc mes deux maisons : le SSM & la Bryo. Merci à la Team du SSM (Annie, Céline, Josie, Marie Catherine & José), Alodie, Nicolas, Philippe, Julien ainsi qu’à tous les étudiants que j’ai pu croiser, pour ces cinq années de bonne humeur, et de joutes culinaires. Merci à l’équipe de Bryologie (Amandine, Marie, Catherine Reeb et Sébastien) pour leur bonne humeur quotidienne et leurs discussions interminables pour faire sortir la Bryologie du côté obscure de la force.

Enfin, je remercie mes parents et ma famille de m’avoir appris et transmit le luxe d’aimer la Nature, de profiter de ceux qui nous entourent, de savourer l’instant présent. Merci.

Sommaire

Liste des Figures

Liste des acronymes

RS : reproduction sexuée

Ras : reproduction asexuée

Ix : insertion de x nucléotides dans la séquence d'ADN

Dy : délétion de y nucléotides dans la séquence d'ADN

Indel : insertion et deletion (de nucleotides)

D. : *Dicranum*

s.l. : sensu lato

s.s. : sensu stricto

Introduction générale

1. Caractéristiques des bryophytes

A- Généralités

Sous le terme bryophytes sont regroupées toutes les plantes terrestres caractérisées par la dominance de la phase haploïde dans leur cycle de vie. Elles se distinguent aussi du reste des *Plantae* par l'absence de lignine véritable et de système vasculaire (absence de racines et de faisceaux conducteurs vrais, à trachéides). On rencontre ces organismes typiquement chlorophylliens (à l'exception de l'espèce *Aneura mirabilis* (Malmb.) Wickett & Goffinet) sur tous les continents.

Les bryophytes constituent un ensemble paraphylétique composé de trois phylums : les *Anthocerotophyta*, les *Marchantiophyta* et les *Bryophyta* différant les uns des autres notamment par l'architecture du gamétophyte et surtout du sporophyte. Il s'agit, en nombre d'espèces, du second groupe de végétaux terrestres, après les Magnoliopsida (Glime, 2007). On considère en effet qu'il existe entre quinze mille espèces (Gradstein & *al.*, 2001) et vingt-cinq mille espèces (Crum, 2001) de bryophytes sur la Terre.

Leur taille est très variable : de quelques millimètres (*Ephemeropsis* K.I. Goebel et *Viridivellus pulchellum* I.G. Stone, Crum 2001) voire moins chez certaines espèces de *Cololejeunea* (Spruce) Schiffner (*C. moramangae* Tixier), à 70 cm de haut chez *Dawsonia superba* Grev. (Crum, 2001*)* jusqu'à 2 m de long chez *Fontinalis* Hedw. (genre aquatique) et 35 m de long chez *Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee var. *lemani* [lac de Genève, Suisse, espèce récoltée à 60 m de profondeur, cf. Husnot, 1884-1890]. De même, le groupe présente une grande amplitude dans ses formes de vie et ses stratégies éco-physiologiques. Le cycle de vie peut s'effectuer en quelques semaines (plantes éphémères, genre *Ephemerum*) ou dans certains cas se réaliser sur plusieurs années (plantes pérennes). En Arctique (station de Swoya, Matsuda), des coussins de *Bryum inconnexum* Cardot ne dépassant pas 5 à 9 cm seraient âgés d'environ cent ans (Longton, 1982). Certaines espèces appartenant au genre *Riccia* L. (Breuil-See, 1994) possèdent des propriétés de reviviscence assez spectaculaires puisque les zones méristématiques restent fonctionnelles après avoir passé un quart de siècle en herbier. De manière plus générale, ces plantes conservent la capacité de déployer leurs feuilles ou leurs thalles par un mécanisme physique lié au pouvoir de réhydratation de leurs parois cellulaires conservé pendant plusieurs décennies.

Les bryophytes se rencontrent dans tous les biotopes terrestres y compris dans des milieux aux conditions contraignantes : toundras, tourbières, hautes montagnes, landes, déserts chauds et froids. Elles marquent le paysage des forêts tropicales humides par leur omniprésence. Certaines espèces tolèrent les eaux saumâtres (*Hennediella heimii* (Hedw.) R.H. Zander, *Riella helicophylla* Bory & Mont.) ou les embruns (*Schistidium maritimum* (Turner ex Scott, Robert) Bruch & Schimp.). Plus surprenant, les bryophytes peuvent croître sur des sols riches en métaux lourds (zinc pour *Scopelophila cataractae* (Mitt.) Broth. et *S. ligulata* (Spruce) Spruce, cuivre et sulfures pour le genre *Mielichhoferia* Nees & Hornsch.). Ces organismes contribuent de façon non négligeable à la structure et au fonctionnement des écosystèmes que ce soit en terme de diversité spécifique, de dynamique végétale, de formation des sols et plus largement dans les cycles biogéochimiques et la régulation des climats. Ainsi, la photosynthèse des bryophytes peut représenter jusqu'à 50 % de la photosynthèse totale d'un écosystème comme par exemple dans les forêts de pin noirs (Delucia & *al.*, 2003, Benscoter & Vitt, 2007). Le genre *Sphagnum*, composant principal des tourbières acides à neutroclines, représente un cas exemplaire de rétention du carbone à long terme. On estime en effet que les 320 Gt de carbone fixé dans les tourbières de l'hémisphère nord équivalent à 44 % du carbone présent sous forme de CO_2 dans l'atmosphère (Rydin & Jeglum, 2006). Ainsi, les sphaignes et leur litière (matériaux tourbeux) représentent une réserve de carbone supérieure à celle des autres plantes (Vanderpoorten & Goffinet, 2009). Les bryophytes jouent également un rôle important dans la rétention de l'eau. Ne représentant en moyenne que 0,5 % de la biomasse totale des forêts tropicales de montagne, elles sont capables de retenir jusqu'à 1 500 % de leur poids en eau (Proctor, 2009). Kürschner & Parolly (2004) et Pypker & *al.* (2006a, 2006b) ont ainsi montré qu'elles peuvent retenir jusque 15 000 kg d'eau par hectare dans les forêts humides tempérées et tropicales.

De même, elles jouent un rôle essentiel dans les dynamiques végétales, dans la formation des sols et des humus, et en constituent des indicateurs sensibles (Lalanne & *al.*, 2008). En particulier elles favorisent et augmentent la dégradation physico-chimique des substrats rocheux, en piégeant les matières organiques et inorganiques. Elles stabilisent et protègent de l'érosion les sols arides en créant des réseaux denses avec les lichens, les champignons et les cyanobactéries (croûte des zones arides). On notera encore leur rôle d'isolant thermique pour le maintien, en été, des sols gelés du type permafrost (Van der Wal & Brooker, 2004). Enfin, de nombreux organismes sont hébergés par ces plantes : myxomycètes des forêts tropicales, aphidies, nématodes, rotifères et tardigrades (Merrifield & Ingham, 1998 ; Peck, 2006). Paradoxalement, les bryophytes sont très peu consommées par

les herbivores excepté dans les régions où elles dominent la végétation (ex. zones arctiques). Leur pouvoir nutritif n'est pas en cause (concentration en minéraux similaire à celle des angiospermes et taux de sucre identique à celle des plantes vasculaires) mais leur digestion est extrêmement lente (Ihl & Barboza, 2007). Ceci s'explique notamment par la présence de composés peu digestes voire inappétents, interprétée comme mécanismes de défense contre l'herbivorie (ex. composés polyphénoliques, métabolites secondaires, terpénoïdes) (Oyesiku & Ogunkolade, 2006). Cependant, certains genres (*Calliergon, Dicranum* et *Polytrichum*) peuvent constituer jusqu'à 40 % du régime alimentaire des lemmings en hiver (Longton, 1992). De même, les capsules immatures d'espèces acrocarpes (*Funariaceae* Schwägr., par exemple) sont consommées par certaines espèces de fourmis, par des orthoptères du genre *Tetrix* ou par des campagnols.

Comme chez 90% des végétaux vasculaires, des relations très étroites existent entre les trois grandes lignées de champignons (*Glomeromycota, Ascomycota & Basidiomycota*) et les bryophytes. Ces interactions, découvertes sur des thalles de *Pellia* Raddi et de *Preissia* Corda (hépatiques) dès 1854 par Schacht, vont du commensalisme au parasitisme en passant par la pathogenèse (voir Read & *al*, 2000 et Davey & Currah, 2006 pour synthèse). Il a ainsi été montré une augmentation du taux de croissance du protonema et de la formation de bourgons chez *Funaria hygrometrica* Hedw. en présence de *Aspergillus niger* Tiegh. et *Alternaria solani* (Ellis & Martin) Jones & Grount., (Hahn & Bopp, 1972). Ces symbioses aboutissent parfois à des échanges de composés organiques entre des plantes vasculaires et des bryophytes via les mycorhizes. Par exemple, au sein des tourbières, *Betula* et *Aneura* échangent du carbone (du premier vers le second, Read & *al.*, 2000) ou bien encore, *Pleurozium shreberi* (Brid.) Mitt. peut fournir du phosphate et du carbone à *Pinus contorta* Dougl. via l'ectomycorhize *Suillus bovinus* (Pres.) Kuntze (Carleton & Read, 1991). Généralement, le mycélium pénètre dans les assises inférieures des thalles ou dans la partie feuillée du gamétophyte ; plus rarement dans le sporophyte. Singulièrement, certaines bryophytes telles que *Plagothecium denticulatum* (Hedw.) Schimp. produisent des substances anti-fongiques contre un large spectre de champignons (Basidiomycètes et Ascomycètes) (Wolters, 1964 ; van Hoof & *al.*, 1981).

Un nombre relativement restreint de bryophytes est capable de tisser des relations épiphytiques ou endophytiques avec des cyanobactéries (Adams & Duggan, 2008 pour synthèse). Le taux de fixation de l'azote des cyanobactéries y est alors bien plus élevé que si elles avaient été libres de toute association (Adams & Duggan, 2008). De fait, ces symbioses ont un rôle non négligeable dans la fixation de l'azote en Arctique, Antarctique et dans les

forêts boréales (Zielke & *al*., 2002, 2005 cité dans Adams & Duggan, 2008) ; régions où les bryophytes représentent une part importante de la végétation (Esseen & *al*., 1997).

Il existe peu de fossiles très anciens appartenant au groupe des *Bryophyta* au sens large. Cependant, des spores arrangées en tétrade dans leur enveloppe protectrice datées de l'Ordovicien (Edwards & *al.*, 1995) ainsi que des microfossiles tubulaires du début du Paléozoïque/Paléozoïque moyen (Graham & *al.*, 2004) permettent d'estimer la présence d'hépatiques sur terre entre 475 et 460 millions d'années (Figure 1).

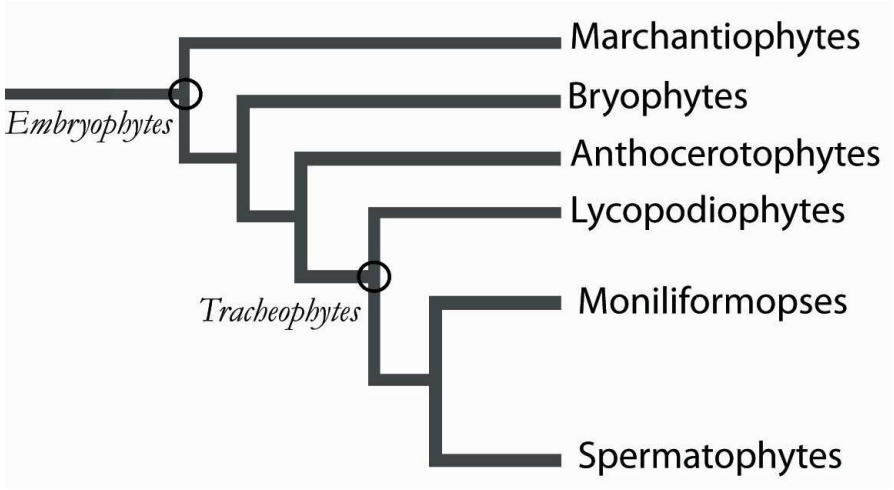

Figure 1 : Relations phylogénétiques entre les grandes lignées de végétaux terrestre (modifié d'après Qui & *al.* , 2006)

B- Systèmes de reproduction

Contrairement aux plantes vasculaires dont le cycle de vie est caractérisé par une alternance haplodiplophasique avec la dominance temporelle et physique de la phase diploïde (2n), les bryophytes sont caractérisées par la dominance de la phase haploïde (n) (Figure 2). Le gamétophyte (n) indépendant, multicellulaire peut être monoïque (plante bisexuée) ou dioïque (plante monosexuée) (Figure 3). Ainsi, approximativement 68 % des hépatiques et 57 % des mousses sont dioïques. La fécondation nécessite le déplacement des spermatozoïdes d'un pied mâle vers un pied femelle. On estime la distance maximale de dispersion des spermatozoïdes de l'ordre d'une dizaine de centimètres (Longton, 1976, 1997; Wyatt, 1982). Ce mode de fécondation, ou zoïdogamie, nécessite la présence d'eau ; les spermatozoïdes flagellés se déplaçant dans le milieu aqueux pour atteindre l'oosphère. Il arrive parfois que les gamètes mâles soient véhiculés par de petits arthropodes (Cronberg & *al*., 2006) ou des mollusques (limaces, escargots…).

En milieu tempéré, le développement des organes reproducteurs se déroule selon la chronologie suivante : (1) les anthéridies (organes reproducteurs mâles) sont initiées en automne/hiver et atteignent leur maturité au printemps/été, leur développement prend plusieurs mois ; (2) les archégones (organes reproducteurs femelles) sont initiés et atteignent leur maturité au printemps/été ; leur développement se déroule sur plusieurs semaines ; (3) la fécondation a lieu en été dans un intervalle de deux semaines à quatre mois (Stark, 2002) ; (4) le sporophyte issu de la fécondation se développe sur le gamétophyte et atteint sa maturité en quelques semaines à quelques mois; (5) les spores, issus de la réduction de méïose dans la capsule, sont alors libérés dans le milieu et dispersés par le vent. Cette durée de maturation plus longue pour les organes mâles augmente la probabilité d'apparition de mutations somatiques chez ces derniers (Stark, 2002). Chez les espèces réalisant l'autofécondation, ce processus expliquerait une part de variabilité génétique.

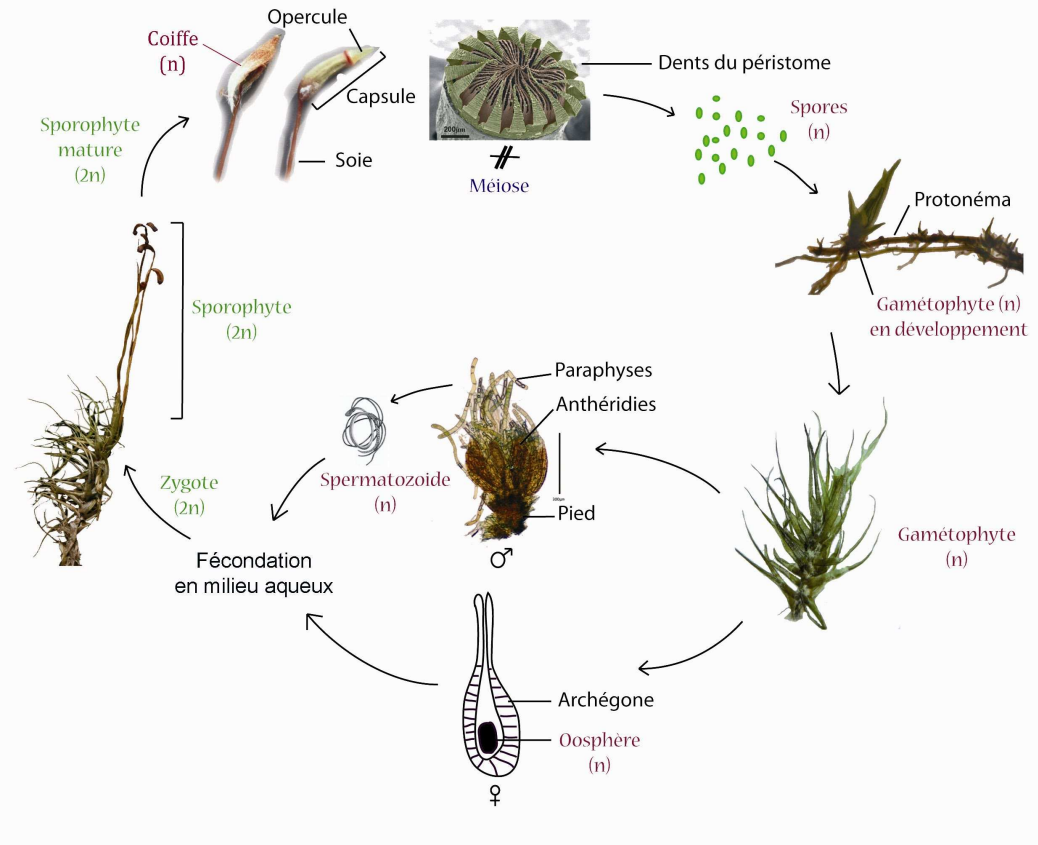

Figure 2: Cycle de vie des bryophytes, exemple du genre *Dicranum*

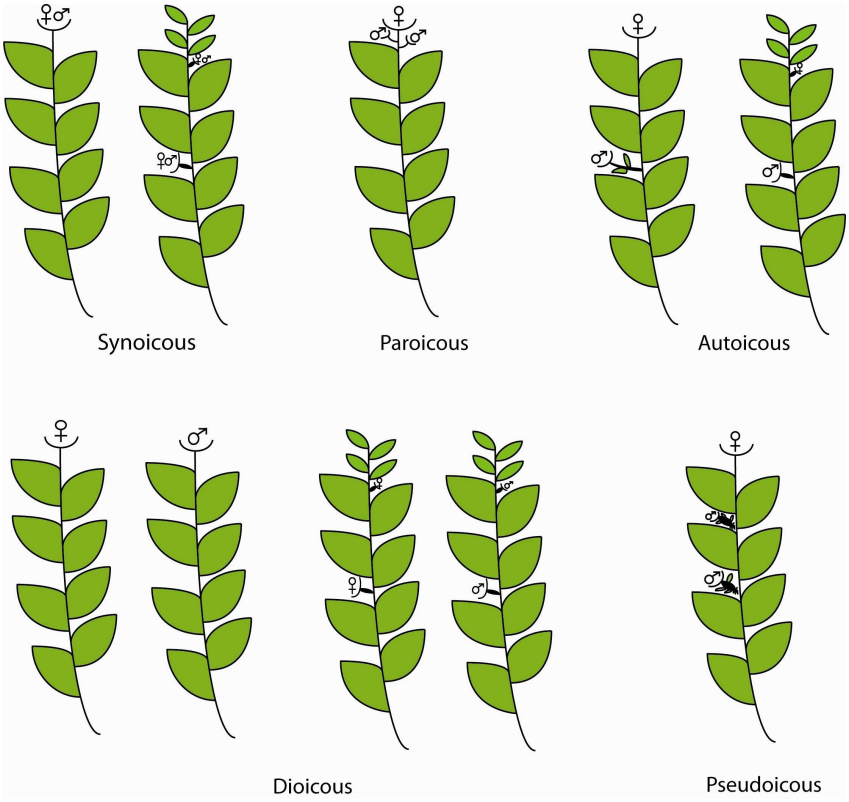

Figure 3 : (A) position relative des organes reproducteurs chez les espèces monoïques, (B) position des organes reproducteurs chez les espèces dioïques

La fécondation et la maturation du zygote, et du sporophyte qui en résulte, suivent six grands patrons chez les plantes à reproduction saisonnière, allant d'une fécondité limitée à une courte période de temps pour les annuelles (60 - 65 jours chez *Funaria hygrometrica* Hedw.) à une fécondité réalisée tout au long de l'année (*Sematophyllum* Mitt. au Japon ou au Brésil) (Stark, 2002).

De manière générale, seule une oosphère par réceptacle est fécondée et conduit à la production d'un sporophyte. Néanmoins, il arrive que plusieurs oosphères d'un même réceptacle soient fécondées aboutissant à la production de plusieurs sporophytes. Ce phénomène est appelé polysétie. Très rare chez les *Marchantiophyta* (un seul cas connu pour un spécimen de *Chiloscyphus cuspidatus* (Nees) J.J. Engel & R.M. Schust.), la polysétie est présente au sein de plusieurs familles de *Bryophyta* comme les *Calymperaceae* Kindb., *Fissidentaceae* Schimp., *Mniaceae* Schwägr., *Polytrichaceae* Schwägr., *Octoblepharaceae* (Cardot) A. Eddy ex M. Menzel, *Dicranaceae* Schimp., ou bien encore chez les *Sphagnaceae* Dumort. (Longton, 1962 ; Egunyomi, 1978).

Les spores unicellulaires sont dispersées dans l'environnement de façon passive (à l'exception de certaines espèces entomophiles appartenant au genre *Splachnaceae* Grev. & Arn.). Aussi, lorsque la capsule arrive à maturité, les variations hygrométriques extérieures entraînent des phénomènes de contraction et de dilatation qui aboutissent à l'ouverture de l'opercule, dévoilant les dents du péristome (Figure 2) (voir Vitt, 1981 pour synthèse). Les spores sont dispersées sur une période allant de plusieurs jours à plusieurs semaines notamment grâce à l'ouverture des dents du péristome. La plupart des spores tombent au sein de la colonie parentale : Miles & Longton (1987) ont ainsi récolté sur une surface de 2 m^2, huit million deux cent mille spores, en 30 jours. Une proportion non négligeable des spores peut atteindre de plus grandes distances (Stoneburner & *al.*, 1992 ; Miles & Longton, 1992). Un dimorphisme de taille des spores peut s'observer chez les bryophytes. On parle alors d'anisosporie (Vitt, 1968).

Plusieurs études montrent directement ou indirectement que le déterminisme sexuel est lié à des chromosomes sexuels (Allen, 1919 ; Vaarama, 1954 ; Ono, 1970 ; Tatuno & Kise, 1970 ; Ramsay & Berrie, 1982 ; Anderson, 1980 ; Shaw & *al.*, 1991 ; McLetchie, 1992). A l'issue de la méiose, on s'attend donc à observer un *sex-ratio* équilibré de spores qui donneront naissance à des gamétophytes mâles et femelles. Or, chez les espèces dioïques, un *sex-ratio* en faveur des plantes femelles a été mis en avant par de nombreuses études (Longton, 1990 ; Wyatt, 1994 ; Bowker & *al*., 2000). Certains mécanismes intervenants à différents stades de la vie des bryophytes expliqueraient ce phénomène : avortement préférentiel des spores mâles (Newton, 1972), germination préférentielle des spores femelles (McLetchie, 1992, 2001), taux de mortalité différent entre les spores (Newton, 1972), taux de croissance différent entre les plantes (McLetchie & Puterbaugh, 2000 ; Newton, 1972), mortalité différente entre les adultes (McLetchie, 1992) et tolérance différente à la dessiccation (Newton, 1972) (Stark (2002).

On notera également que le coût de la reproduction diffère entre les espèces monoïques et les espèces dioïques. Chez les gamétophytes unisexués, la fonction mâle demande un investissement pré-zygotique plus élevé que la fonction femelle. Cela se comprend si l'on considère (1) que les spermatozoïdes sont riches en lipides (Paolillo, 1979), (2) que les anthéridies sont initiées plus tôt que les archégones, (3) qu'elles mettent plus de temps à atteindre leur maturité et qu'elles nécessitent donc un investissement énergétique plus important (les anthéridies sont produites trois mois plus tôt que les archégones chez *Atrichum rhystophyllum* (C. Müll.) et *Pogonatum inflexum* (Lindb.) Lac. (Imura, 1994)). Qui plus est, il y a de très nombreux spermatozoïdes par anthéridies alors qu'il n'y a qu'une oosphère par

archégone (trois fois plus d'anthéridies par périgonium[1] que d'archégones par périchétium[2] chez *Atrichum rhystophyllum* (Müll. Hal.) Paris et *Pogonatum inflexum* (Lindb.) Lac. (Imura, 1994 ; Stark, 2002).

De fait, le succès de la reproduction sexuée, exprimé par le pourcentage de production de sporophytes, est plus faible chez les espèces dioïques. Chez ces dernières, outre les hypothèses liées au *sex-ratio*, il faut également prendre en compte (1) l'existence de populations unisexuées ou de ségrégation spatiale (chez *Splachnum sphaericum* Hedw. Cameron & Wyatt, 1990 ; chez *Syntrichia caninervis*, Bowker & *al.* 2000), (2) la courte distance de dispersion des spermatozoïdes, (3) la différence de croissance et de mortalité entre les sexes (McLetchie & *al.*, 2002), (4) l'inhibition dans la formation des gamétanges chez un sexe, et (5) les différences de fécondité entre mâle et femelle (Bowker & *al*., 2000).

Par ailleurs, il a été montré, chez les bryophytes, l'existence d'un dimorphisme sexuel qui s'exprime de différentes façons : anisosporie (voir § spores, p.15), mâles nains (Loveland, 1956 ; Ramsay, 1979 ; Patterson & *al.*, 1998) ou élongation des tiges (Imura, 1994). Présents dans trente-sept familles de *Bryopsida* (ex. *Ptychomniaceae* M. Fleisch., *Dicranaceae* Schimp), les mâles nains, dont la taille varie de quelques millimètres à quelques centimètres, sont toujours épiphytes des pieds femelles. Selon les cas, ils apparaissent être strictement nains (ex. *Dicranum polysetum* Sw.) ou avoir une taille variable, suivant la distance à la plante femelle (*Leucobryum glaucum* (Hedw.) Ångström, *Dicranum scoparium* Hedw.) (Loveland, 1956 ; Patterson & *al.*, 1998). Chez *Atrichum rhystophyllum* et *Pogonatum inflexum*, les tiges mâles croissent également plus tôt dans l'année et plus lentement que les tiges femelles (Imura, 1994).

La localisation spatiale de la fonction mâle par rapport à la fonction femelle implique trois cycles de vie possibles (Figure 4) aux conséquences variables sur la diversité génétique des descendants. Les bryophytes peuvent réaliser (1) de l'autofécondation en état monoïque (état A) mais aussi dioïque (état B) d'autant qu'aucun cas d'incompatibilité pré- ou post-zygotique n'est connu à ce jour chez ces organismes, ou bien elles peuvent réaliser (2) de l'allofécondation (état C).

[1] Structure de reproduction sexuée regroupant les organes mâles, plusieurs périgonium peuvent être présent sur un pied mâle

[2] . Structure de reproduction sexuée regroupant les organes femelles, plusieurs périchétium peuvent être présent sur un pied femelle

Les études qui s'intéressent à ce sujet montrent que de nombreuses bryophytes ont un système de reproduction sexué mixte avec des proportions variables d'auto et d'allofécondation (Shaw, 2001). Par ailleurs, chez certaines espèces telles que *Leptodontium gemmascens* (Mitt.) Braithw. ou *Syntrichia rigescens* (Broth. & Geh.) Ochyra, les organes sexuels n'ont jamais été observés.

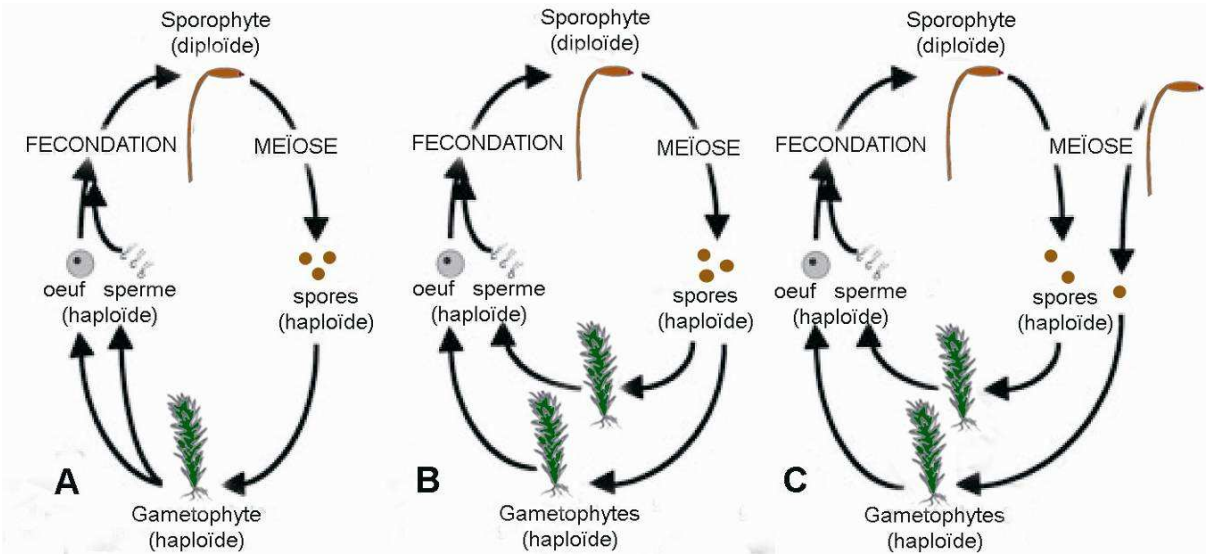

Figure 4 : Cycle de vie haplodiplophasique des bryophytes modifié d'après Crawford & al. (2008) (A) espèce monoïque, (B, C) espèce dioïque

En outre, les bryophytes peuvent réaliser, simultanément ou non, de la reproduction sexuée et de la multiplication végétative à travers un certain nombre de structures plus ou moins spécialisées (Figure 5). Toutes les bryophytes sont capables de réaliser de la reproduction végétative (McLetchie & *al.*, 2002) de façon continue ou discontinue au cours de la vie du gamétophyte. Un grand nombre d'organes plus ou moins spécialisés sont dévolus à la propagation végétative (cf. classification de Newton & Mischler, 1994 et de Laaka-Lindberg & *al*., 2003) qui joue un rôle important dans le maintient des populations locales de bryophytes.

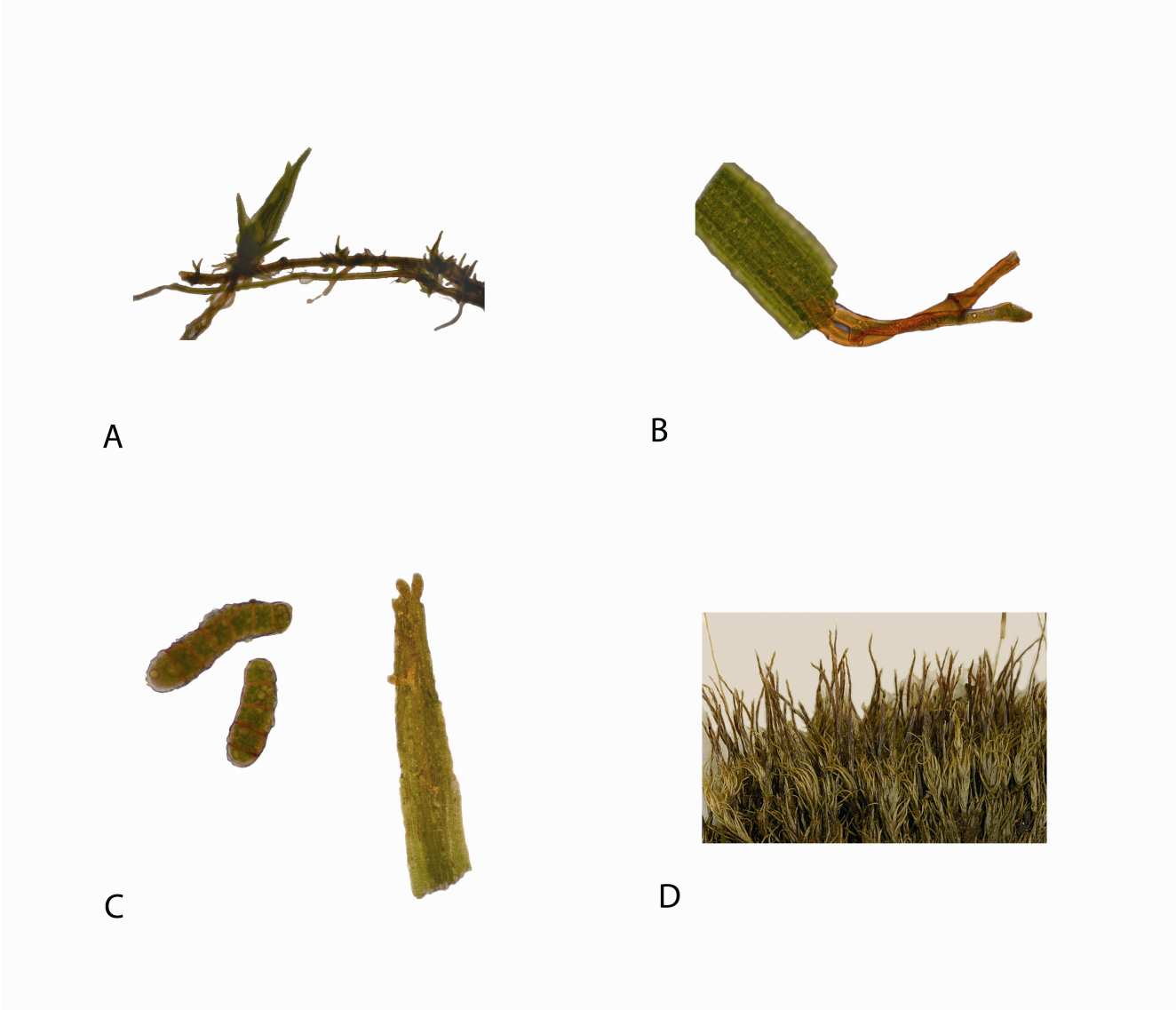

Figure 5 : Structures de reproduction végétative (A) germination d'un protonéma, (B) bris de feuille germant chez *D. viride*, (C) gemmules chez *D. tauricum*, (D) branches flagelliformes chez *D. flagellare*

C- Dispersion et variabilité

La dispersion ou action de disperser est un mécanisme actif ou passif, visant à disséminer sa descendance (spores) ou tout ou partie de soi (gemmules, bulbilles, bris de feuilles, branches flagelliformes, cladies ou sperme…) à travers des diaspores sexuées et asexuées. Elle est, de fait, directement liée à la production de ces diaspores et donc à la stratégie reproductive de l'espèce.

La plupart des plantes se reproduisent par voix sexuée et asexuée et le ratio d'une stratégie par rapport à l'autre varie entre les espèces et à l'intérieur d'une même espèce (Zhang & Zhang, 2007). Chez les bryophytes, outre la reproduction sexuée, toutes les espèces

sont capables de se disperser végétativement (McLetchie & *al.,* 2002). Ces deux modes de reproduction nécessitent de l'énergie et un compromis s'établit nécessairement au sein de la plante (Zhang & Zhang, 2007). Les coûts et les bénéfices d'une stratégie par rapport à l'autre voudraient que la reproduction sexuée soit favorisée pendant les stades de colonisation et la reproduction asexuée soit favorisée au sein des colonies matures (Eckert, 2002), et que la dispersion par spores et la dispersion par propagules asexuées soient respectivement favorisées.

La dispersion des bryophytes est principalement réalisée par le vent, mais d'autres vecteurs tels que les animaux (limaces, chauves-souris, fourmis, ou mouches ; Parsons & *al.* 2007 ; Rudolphi, 2009 ; Kimmerer & Young, 1995, Goffinet & *al*., 2004), sont également connus comme mécanismes de dispersion. Néanmoins, la seule présence d'un agent de dispersion ne garantit pas la propagation des diaspores. La durée de leur pouvoir germinatif et leur tolérance à la dessication contraignent également leur pouvoir dispersif. Van Zanten (1978) a ainsi montré qu'il existait une corrélation entre la capacité de dispersion liée à la résistance à la dessication des spores et l'aire de distribution des espèces tempérées.

Selon les capacités de dispersion des diaspores, on parlera de dispersion à courte distance (DCD : quelques millimètres à quelques mètres) ou de dispersion à longue distance (DLD : plusieurs milliers de kilomètres, Van Zanten, 1978). Les structures mises en jeu ainsi que les conséquences biologiques et évolutives seront différentes si l'on est en DCD ou en DLD (ex. 80 % des gemmules d'*Anastrophyllum hellerianium* tombent dans les 50 cm autour de la colonie dont ils sont issus (Pohjamo & *al.,* dans McLetchie & *al.,* 2002). En effet, chez les espèces dioïques (sexes séparés), la reproduction végétative aura d'autant plus de conséquences qu'elle va modifier l'accès à un partenaire sexuel en augmentant la distance à ce dernier. Le taux de reproduction sexuée et, conséquemment, la dispersion à longue distance via les spores, vont alors diminuer. A l'extrême, le *sex-ratio* pourra être localement biaisé jusqu'à rendre les populations monosexuées (Longton & Schuester, 1983). Ceci est d'autant plus vrai que les diaspores issues de la reproduction végétative sont disséminées sur une plus faible distance que les diaspores sexuées car elles manquent de mécanisme de dispersion active et de mécanisme de dormance, bien qu'elles soient généralement plus grosses que les diaspores sexuées (Eckert, 2002).

Le flux de gène résultant de cette dispersion (ou migration) est un facteur évolutif important qui peut augmenter ou diminuer la variabilité génétique à l'intérieur des populations selon qu'elles étaient originalement génétiquement proches ou non (Korpelainen & *al.,* 2005). Par ailleurs, ce trait d'histoire de vie a de profondes conséquences pour les

populations. D'un point de vue écologique, la dispersion influence la dynamique et la persistance des populations, la distribution et l'abondance des espèces ainsi que la structure de la communauté. D'un point de vue évolutif, la dispersion détermine le flux de gène entre les populations et affecte des processus tels que l'adaptation locale, la spéciation et l'évolution des traits d'histoire de vie (Diekmann & *al*., 1999).

2 Objectifs de la thèse

A- Choix du modèle d'étude

A l'origine, ce travail est à replacer dans un cadre général, se proposant de tester un certain nombre d'hypothèses et de répondre à des questions portant sur l'évolution des systèmes de reproduction. Il s'appuie sur une plante modèle pertinente, dont le choix est justifié ci-après. Ces hypothèses sont les suivantes : (1) la reproduction sexuée (RS) apporte plus de diversité génétique que la reproduction asexuée (Ras), (2) la RS permet d'accroître la vitesse de l'adaptation et évite l'accumulation des mutations délétères, (3) les espèces à reproduction asexuée sont phylogénétiquement plus récentes que les espèces à reproduction sexuée, (4) les espèces monoïques ont des taux de fécondité plus élevés que les espèces dioïques, (5) pourquoi chez les espèces dioïques la sexualité est d'autant plus réduite que la plante vit dans un environnement extrême ? (6) les patrons d'allocation des ressources pour les pieds mâles et les pieds femelles sont-ils les mêmes chez les espèces monoïques que chez les espèces dioïques ? (7) quels sont les compromis entre RS et Ras ? En effet, très peu d'études concernant l'évolution des systèmes de reproduction chez les végétaux terrestres considèrent les bryophytes comme modèles d'études (Smith, 1978 ; During, 1979 ; Longton & Schuster, 1983 ; Wyatt & Anderson, 1984) et seule l'étude de Crawford & *al,* (2008) y intègre le facteur phylogénétique. De fait, les hypothèses qui ont été proposées jusqu'alors sont essentiellement fondées sur des données empiriques issues du monde des végétaux vasculaires. Il s'agissait donc de savoir si l'évolution des systèmes de reproduction chez les bryophytes obéit aux mêmes règles et présente les mêmes conséquences que chez les plantes vasculaires.

Il nous fallait choisir un genre au sein duquel différents patrons de reproduction s'exprimaient : reproduction sexuée et asexuée, monosétie et polysétie, présence et absence de dimorphisme sexuel, espèces monoïques et dioïques. Aucun genre ne possède toutes ces caractéristiques ; cependant le genre *Dicranum,* présent en France métropolitaine constitue un très bon modèle d'étude bien qu'il n'offre que des espèces dioïques. Des études moléculaires

préliminaires réalisées chez *Dicranum viride* (Sull. & Lesq.) Lindb. et chez *Dicranum scoparium* dans le cadre de stages de master (Pichonet, 2006 et 2007) ayant déjà permis la mise au point d'un protocole d'échantillonnage et la récolte de spécimens de différents pays européens ainsi que la mise au point de marqueurs moléculaires, ont appuyé le choix de ce groupe d'étude.

Ces considérations évolutives nécessitent un cadre phylogénétique bien établi pour le genre et idéalement pour la sous famille des *Dicranoïdeae*. Or actuellement, seules des données morphologiques nous permettent d'inférer les relations de parenté éventuelles entre les espèces du groupe. C'est pourquoi, un travail de phylogénie moléculaire a été mis en place, parallèlement à l'analyse des caractères morphologiques. La taxinomie a nécessité un réexamen des caractères morphologiques et une remise en question de la pertinence de certains des caractères présentés comme discriminants, à partir d'une littérature trop souvent limitée à des études régionales, pour un groupe présent sur tous les continents. Cette première étude des relations de parenté au sein du genre *Dicranum* offre alors un cadre phylogénétique fiable permettant de tester les hypothèses citées ci-dessus.

B- Buts de la thèse

Le genre *Dicranum* connu pour sa diversité spécifique, sa large répartition tout autour du globe mais surtout pour la plasticité phénotypique d'un grand nombre de ses espèces, reste encore mal circonscrit. Le nombre de taxons varie de 60 à plus de 150 suivant les ouvrages, les auteurs et les révisions taxinomiques. De même, un certain nombre d'espèces ne sont connues qu'à travers le spécimen type et la diagnose *princeps* est souvent succincte et peu informative. De nombreux bryologues ont procédé à des analyses morphologiques. Malheureusement elles sont souvent limitées à des taxons appartenant à une zone géographique donnée (ex. Bellolio-Trucco & Ireland, 1990 pour l'Ontario et le Québec ; Takaki, 1964 pour le Japon ; Cao & Tao, 1992 pour la Chine, Crum, 1981 pour l'est de l'Amérique du Nord). Il n'existe donc ni d'étude synthétique récente portant sur la morphologie du groupe ni aucune donnée moléculaire.

Ce travail se propose de répondre aux questions suivantes :

(i) Quelle est la diversité spécifique du genre *Dicranum* ?

(ii) Quelle est la variabilité morphologique et moléculaire de ses espèces ?

(iii) Quelles sont les relations phylogénétiques des espèces congénériques ?

(iv) Existe t-il des patrons géographiques au sein de ce genre ?

Pour répondre à ces questions, il est nécessaire de disposer du plus grand nombre possible de taxons représentatifs non seulement du genre mais aussi de la distribution géographique de chacun d'entre eux. Des missions sur le terrain ont été effectuées et plusieurs collaborations ont été mises en place avec des bryologues de différents pays afin de disposer de matériel récent compatible avec des analyses moléculaires. Une fois cet échantillonnage acquis, des analyses combinant recherches bibliographiques, études morphologiques et analyses moléculaires ont été entreprises. Les collections du Muséum national d'histoire naturelle renferment un très grand nombre de types appartenant au genre *Dicranum* ; et l'établissement dispose de toute la littérature qui s'y rapporte. Ceci a facilité et a permis un réexamen approfondi des caractères morphologiques au cours de ces trois ans de recherches.

(1) Le premier chapitre a pour objectif (i) de faire un état de l'art sur la connaissance du genre (morphologique, écologie, cycle de vie), et (ii) de réaliser la première phylogénie moléculaire du genre *Dicranum*,

(2) Le second chapitre s'intéresse plus particulièrement à l'analyse moléculaire d'une espèce circumpolaire : *D. majus,* dans le but de tester un patron morphologique récemment mis en évidence en Europe,

(3) Le troisième chapitre argumente des considérations évolutives sur les systèmes de reproduction (mâles nains) et des considérations taxinomiques sur quelques espèces,

En conclusion, les résultats obtenus lors de ces travaux sont mis en perspective

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Chapitre I: Circonscription du genre *Dicranum* Hedw.: approches morphologique et moléculaire

No one definition has as yet satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species.

Darwin

Dicranum fait partie des quarante et un genres constitutifs de la famille des Dicranaceae (Bryopsida). Etablit par Hedwig en 1801, son nom fut choisit en référence au péristome composé de dents bifides (**Figure 6**) (du grec *dicranon* = fourche). Hedwig reconnut trente quatre espèces de *Dicranum* dont cinq sont encore actuellement valides, parmi lesquelles *Dicranum scoparium* Hedw., l'espèce type. Il divisa le genre en deux sections : section *pedunculis rectis* (27 espèces) et section *pedunculis inflexis* (7 espèces), selon la forme de la soie, droite dans la section *rectis* et flexueuse dans la section *inflexis*.

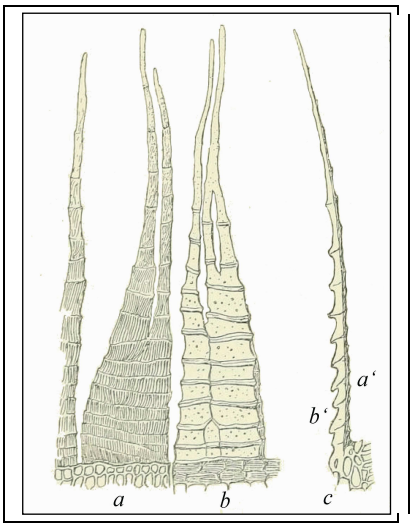

Figure 6: Structure typique des dents de péristome chez le genre *Dicranum*, (a) surface externe, colorée, fine, densément striée verticalement vue de face, (b) surface interne, plus épaisse, souvent papilleuse, avec des bandes horizontales saillantes vue de face, (c) coupe de dent de péristome avec (a') surface externe et (b') surface interne (échelle :100ème), d'après Fleischer (1900-1922)

La multitude d'espèces décrites sous le nom de *Dicranum*, qui inclut 885 binômes valides (van der Wijk & *al.*, 1962 ; www.tropicos.org), nous empêche de faire une revue exhaustive de l'histoire du genre. Différents concepts taxonomiques ont été utilisés depuis sa description, certains considérant notamment les formes de croissances différentes comme des espèces proprement dites. De plus, l'absence de connaissances sur l'amplitude de la plasticité phénotypique des espèces a abouti à la description d'une même entité biologique sous pléthore de noms différents à travers le monde. On dénombre, par exemple, 18 synonymes pour *D. scoparium* lui-même subdivisé en 56 variétés ou bien 13 synonymes pour *D. bonjeanii* De Not lui-même subdivisé en 22 variétés (www.tropicos.org). Parallèlement, quatre-vingt une sections ou variétés ont été décrites depuis 1801 afin de regrouper les espèces par affinité morphologique. Certaines de ces sections, au nombre de quinze, furent intégrées puis retirées ultérieurement pour être érigées au rang de genre à part entière (ex. *Campylopus* Brid., *Dicranodontium* Bruch. & Shimp., *Kiaeria* I. Hagen, *Arctoa* Bruch. &

Schimp.) et dix-huit sections initialement décrites sous *Dicranum* ont été, par la suite, également érigées au rang de genre à part entière (ex. *Pseudochorisodontium* (Broth.) C. Gao & *al*., *Oncophorus* (Brid.) Brid., *Chorisodontium* (Mitt.), *Paraleucobryum* (Lindb. ex Limpr.) Loeske, *Dicranoloma* (Renauld) Renauld*)*. Le Tableau 1 synthétise les caractères morphologiques discriminant ces genres. Actuellement, quarante-neuf sections sont valides (www.tropicos.org). Cependant, la plupart de ces classifications infragénériques ne peuvent être appliquées à l'ensemble des espèces constitutives du genre sans devoir en élargir les définitions ou créer de nouvelles sections (Klazenga, 1999).

A présent, cent trente-deux espèces sont acceptées sous le nom de *Dicranum* (compilation bibliographique d'après van der Wijk & *al.*, 1962 et www.tropicos.org; Tableau 1). Le genre, circonscrit exclusivement sur des bases morphologiques, se distingue de ses groupes frères par un faisceau de preuves (Tableau 1). Les principaux caractères sont: 1) des feuilles droites à falciformes avec une nervure étroite (< 1/3 de la largeur de la feuille à la base), 2) des cellules du limbe lisses ou mammilleuses et souvent poreuses, 3) des cellules alaires différenciées, généralement unistratifiées, 4) une nervure souvent rugueuse dorsalement, 5) des capsules droites ou inclinées, striées ou pliées lorsqu'elles sont sèches. Comme ses groupes proches, le genre *Dicranum* est dioïque et peut être polysétique (plusieurs oosphères sont fécondées dans un périchétium). La plupart des espèces sont reconnues sur la base de la taille et la forme des feuilles, la taille et la forme des cellules ainsi que la structure et la forme de la nervure. Cependant, certaines espèces peuvent être très plastiques (voir aussi Briggs, 1965), par exemple, *D. scoparium* est considéré comme le taxon le plus polymorphique du genre pour l'Amérique du nord, avec des feuilles qui varient d'une forme typiquement lancéolée et longuement acuminée à une forme ovale lancéolée et courtement acuminée (Moss Flora of North America, www.tropicos.org). L'utilisation de caractères discriminants sujets à plasticité (ex. forme ou taille de la feuille, présence de plus ou moins de dents sur la marge de la feuille) combinée à des révisions taxinomiques essentiellement régionales (ex. Briggs, 1965; Bellolio-Trucco & Ireland, 1990; Takaki, 1964, 1972; Gao & Cao, 1992) et l'absence de révision mondiale en font un genre complexe.

Tableau 1: Caractères morphologiques d'anciennes sections du genre *Dicranum* érigées au rang de genre, compilé d'après Allen & Ireland (2002), Allen (1989, 1990, 1997), Crum & Anderson (1981), Frahm (1989, 1997), Frahm & *al.* (1998), Gao & *al.* (1999), Müller & Frahm (1987) et Limpricht (1890), Klazenga (1999, 2003)

	Dicranaceae					
	Dicranoideae					
	Dicranum Hedw.	*Paraleucobryum* (Lindb. ex Limpr.) Loeske	*Pseudochorisodontium* (Broth.) C. Gao et *al*.	*Chorisodontium* (Mitt.) Broth.	*Holomitrium* Brid.	*Dicranoloma* (Renauld) Renauld
Nervure	largeur < 1/3 de la base de la feuille, souvent striée et dentée dorsalement, une couche de cellules guides avec deux bandes de stéréïdes, absence de couche ventrale de hyalocystes	largeur 1/3-2/3 de la base de la feuille, lisse ou striée dorsalement, absence de stéréïdes, 3-4 couches de hyalocystes entremêlés avec les chlorocystes	étroite, lisse ou faiblement dentée dorsalement, non striée, absence de couche ventrale de hyalocystes	excurrente, une couche de cellules guides avec plusieurs couches de stéréïdes ventrales et dorsales	lisse dorsalement ou avec quelques dents à l'apex, absence de couche ventrale de hyalocystes	mince à relativement robuste (20 - 160 µm de large); subpercurente à excurrente, lisse ou faiblement dentée dorsalement, 2 – 14 couches de cellules guides, 1 – 4 couches de stéréïdes dorsales, absence de couche ventrale de hyalocystes
Cellules alaires **Nombre de couches**	souvent dilatées, généralement jaunes-brunâtres près de la marge et hyalines près de la nervure, 1-2 (3-4) couches	dilatées, 1-2 couches	dilatées, rougâtres-brunes, 2–5(–6) couches	dilatées	dilatées, 1 couche	différenciées, 1 couche de petites cellules sub-carrées *marge du limbe différentiée
Tige, en section	groupe central présent	groupe central faible	groupe central présent	groupe central faible	groupe central présent	groupe central présent ou absent
Soie	droite à l'état humide	droite à l'état humide	droite à l'état humide	allongée, droite assez robuste	droite à légèrement incurvée	droite, solitaire or agrégée
Feuilles périchétiales	convolutées, généralement différenciées	embrassantes, parfois subulées avec une base ovale	fortement embrassantes à la base, se rétrécissant abruptement en un acumen court ou long	convolutées, embrassantes, se rétrécissant abruptement en un acumen court	convolutées, très longues et étroites atteignant ou dépassant la capsule	différenciées; feuilles externes ovales à fortement ovales, feuilles internes elliptiques à fortement ovales

						lancéolées devenant graduellement acuminées
Capsule	droite, inclinée ou horizontale, allongée à cylindrique, plus ou moins ridé à sec	droite, cylindrique, symétrique	droite, cylindrique, symétrique	droite légèrement asymétrique, courte à longuement cylindrique, lisse	droite, ovoïde - courte à longuement cylindrique	droite ou arquée, ovoïde à cylindrique, strumeuses
Opercule	longuement rostré	longuement rostré	très longuement rostré (aussi long ou presque aussi long que la capsule)	rostré	rostré	long, conique, rostré
Stomates	présents	présents	absents	phaneroporic	phaneroporic	présents
Dents du péristome	bifides à 1/2-2/3, verticalement striées, dans la partie inf. légèrement papilleuses	bifides à ½, striées de façon oblique ou croisées, dans la partie inf. légèrement papilleuses	absentes ou fortement réduites	divisées irrégulièrement (mais parfois entière) verticalement striées, papilleuses ou non au sommet	divisées irrégulièrement (parfois entière) à plus ou moins fenêtrées, généralement papilleuses	profondément divisées, en dessous de la division souvent fenêtrées, partie sup. papilleuse
Coiffe	surface lisse, base entière	cucullée, surface lisse, base entière	base entière	cucullée, lisse	cucullée, surface lisse, base entière	cucullée, entière à légèrement déchiré sur les marges inférieures.
Rhizoides	prennent toujours naissance sur la tige ou à la base des branches, jamais de la nervure	naissant parmi les feuilles	inconnus	inconnus	inconnus	naissant en dessous de l'insertion des feuilles

Tableau 1 (suite)

	Rhabdoweisiaceae			*Leucobryaceae*	
	Kiaeria I. Hagen	*Arctoa* Bruch & Schimp.	*Oncophorus* (Brid.) Brid.	*Campylopus* Brid.	*Dicranodontium* B.S.G.
Nervure	généralement excurrente, étroite, stéréïdes peu différenciées des cellules guides, absence de couche ventrale de hyalocystes	excurrente, étroite, stéréïdes peu différenciées des cellules guides, absence de couche ventrale de hyalocystes	forte, percurrente à légèrement excurrente, étroite, avec 2 bandes stéréïdes, absence de couche ventrale de hyalocystes	largueur 1/3 à 7/8 de la base de la feuille, plus ou moins striée et dentée dorsalement, stéréïdes dorsales bien développées, couche ventrale hyalocystes présente	largueur 1/3 à ½ de la base de la feuille, plus ou moins rugueuse dorsalement dans la partie sup., avec deux couches de stéréïdes, absence de couche ventrale de hyalocystes
Cellules alaires **Nombre de couches**	bien différenciées, généralement brunes	dilatées, parfois différenciées	parfois légèrement élargies ou légèrement différenciées	généralement nettement dilatées, hyalines à brunes rougeâtres, 1(2) couches	plus ou moins dilatées, hyalines ou brunâtres, une couches
Tige, en section	groupe central présent	groupe central faiblement differencié	groupe central présent	groupe central présent	groupe central présent
Soie		droite à l'état humide, stout, solitaire	droite à l'état humide, tordue - sinueuse à l'état sec	courbée à l'état humide, parfois droite – flexueuse	fortement courbée à géniculée l'état humide, droite - sinueuse à l'état sec
Feuilles périchétiales	embrassantes à la base	embrassantes longuement acuminées	peu différenciées	différenciées ou non	souvent avec une base plus large et longue qui se rétrécie plus abruptement que les feuilles de la tige
Capsule	sub-droite, courbée, cylindrique, lisse à plissée à l'état sec, indistinctement strumeuse	droite, symétrique, obovoïde, émergée ou immergée dans les feuilles périchétiales, resserrée en dessous de la bouche, plissée à l'état sec	inclinée à horizontale, arquée, cylindrique, resserrée en dessous de la bouche, plissée à l'état sec, distinctement strumeuse	droite ou courbée, ovoïde à ellipsoïde, lisse à fortement plissée à l'état sec	droite, ovoïde - cylindrique, lisse à faiblement plissée à l'état sec
Opercule	rostré de façon oblique	rostré de façon oblique	rostré, arqué	rostré	très longuement rostré (aussi long ou presque aussi long que la capsule)

Stomates				absents	absents
Dents du péristome	à moitié bifides, papilleuses à striées verticalement ou ponctuées striées	à moitié bifides, verticalement ou irrégulièrement striées	à moitié bifides, verticalement ponctuées striées à la base, papilleuses distalement	bifides à ½, partie externe généralement striées, partie interne généralement papilleuse	bifides presque jusqu'à base
Coiffe	cucullée, occasionellement rugueuse à l'apex	cucullée, lisse	cucullée, lisse	cucullée, surface lisse base généralement bordée par des poils unicellulaires ou parfois bordée par des longs poils généralement caducs	cucullée, surface lisse, base entière ou rarement cilliée
Rhizoides	inconnus	inconnus	inconnus	prennent généralement naissance à la surface dorsale de la nervure	naissent des deux côtés de la nervure, parmi les tiges et la base des branches

Tableau 2: Liste actualisée des espèces du genre *Dicranum* basée sur la synthèse bibliographique d'après van der Wijk & *al.* (1962) et www.tropicos.org. Les zones géographiques sont définies d'après van der Wijk & *al.* (1962), (*) espèces connues uniquement à travers leur spécimen type ; (Na) mâle nain, (No) mâle de taille normale, (Nr) non renseigné ; (M) monosétique, (P) polysétique, (Nr) non renseigné ; (G) gemmules, (Bfl) branches flagelliformes, (B) bris de feuilles

	Espèce	Aire de distribution	Taille des pieds mâles Na, No, Nr	Type de sétie M, P, Nr	Organes de reproduction végétative G, Bfl, B, Abs, Nr	Référence bibliographique originale
1	*Dicranum acanthoneurum* Müll. Hal.	afr2	Nr	Nr	Nr	Flora 73: 474. 1890
2	*Dicranum acutifolium* (Lindb. & Arnell) C.E.O. Jensen	eur, as1, am1	Na	M	Abs	Förteckning över Skandinaviens Växter. 2. Mossor (Andra Upplagan) 18. 1937
3	**Dicranum alpinum* (P. Beauv.) Brid.	eur, as1, am1	Nr	Nr	Nr	Muscologia Recentiorum Supplementum 1: 208. 1806.
4	*Dicranum arcuatipes* Müll. Hal.	austr2	Nr	Nr	Nr	Genera Muscorum Frondosorum 299. 1900
5	*Dicranum assamicum* Dixon	as3	No	M	Abs	Journal of the Bombay Natural History Society 39: 774. 1937
6	*Dicranum atratum* Geh.	as1	Nr	Nr	Nr	Flora 62: 473. 1879
7	**Dicranum atro-viride* Cardot	am6	Nr	Nr	Nr	Annales Botanici Societatis Zoologicae-Botanicae Fennicae "Vanamo" 9: 41. 1937
8	**Dicranum beyrichianum* (Duby) Hampe	am5	Nr	Nr	Nr	Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn 9-10: 255. 1878
9	*Dicranum bonjeanii* De Not.	eur, as1, am1	Na, No	M, P	Abs	Elenco dei Muschi 29. 1837
10	*Dicranum borbonicum* Renault & Cardot	afr3	Nr	Nr	Nr	Prodrome de la Flore Bryologique de Madagascar des Mascareignes et des Comores 160. 1898
11	*Dicranum braunsiae* Müll. Hal.	as2	Nr	Nr	Nr	Genera Muscorum Frondosorum 290. 1900
12	*Dicranum brevifolium* (Lindb.) Lindb.	eur, as1, am1	Na	M	Abs	Musci Scandinavici 24. 1879
13	*Dicranum caesium* Mitt.	as2	Nr	Nr	Nr	Transactions of the Linnean Society of London, Botany 3: 156. 1891
14	**Dicranum caldense* Mümm. Hal.	am5	Nr	Nr	Nr	Hedwigia 39: 250. 1900
15	**Dicranum capillatus* (Hook. & Wilson) L.C. Beck	austr1	Nr	Nr	Nr	Transactions and Proceedings of the New Zealand Institute 25: 301. 1893
16	**Dicranum carneum* Blandow	eur	Nr	Nr	Nr	Deutschlands Flora, Abtheilung II,

						Cryptogamie 10: ic.. 1809
17	*Dicranum cheoi* E.B. Bartram	as2	Na	M	Abs	Annales Bryologici 8: 8. f. 3. 1936
18	**Dicranum clericii* Brizi	am	Nr	Nr	Nr	Bollettino de Società Geologica Italiana 9: 365. 1892
19	**Dicranum columbiae* (Kindb.) Renauld & Cardot	am1	Nr	M	Nr	Revue Bryologique 19: 77. 1892
20	*Dicranum condensatum* Hedw.	am1	Na	M	Nr	Species Muscorum Frondosorum 139. 34 f. 6-10. 1801
21	**Dicranum conglomeratum* (Brid.) Wallr.	eur	Nr	M	Nr	Flora Cryptogamica Germaniae 1: 169. 1831
22	**Dicranum craigieburnense* R. Br. Bis	austr2	Nr	Abs	M	Transactions and Proceedings of the New Zealand Institute 29: 457. 1897
23	*Dicranum crassifolium* Sérgio, Ochyra & Seneca	eur	Na	M	Abs	Fragmenta Floristica et Geobotanica 40: 204. f. 1-4. 1995
24	*Dicranum crispatulum* (Roll.) Kindb.	am1	No	Nr	Nr	European and N. American Bryineae (Mosses) 2: 189. 1897
25	*Dicranum crispifolium* Müll. Hal.	as2, as3	Nr	M	Nr	Botanische Zeitung (Berlin) 22: 349. 1864
26	*Dicranum decumbens* Thwaites & Mitt.	as3	Nr	Nr	Nr	Journal of the Linnean Society, Botany 13: 296. 1873
27	*Dicranum deflexicaulon* Müll. Hal.	am4	Nr	M	Nr	Linnaea 38: 589. 1874
28	*Dicranum delavayi* Besch.	as2	Nr	M	Nr	Revue Bryologique 18: 88. 1891
29	*Dicranum dilatinerve* Cardot & P. de la Varde	as3	Nr	Nr	Nr	Revue Bryologique 49: 35. 1922
30	*Dicranum diplospiniferum* C. Gao & C. W. Aur	as2	Nr	Nr	Nr	Bulletin of Botanical Laboratory of North-Eastern Forestry Institute 7: 99. 1980.
31	*Dicranum dispersum* Engelmark	eur	Na	Abs	M	Stuttgarter Beiträge zur Naturkund. Seria A, Biologie 592: 4. f. 1-3. 1999
32	*Dicranum drummondii* Müll. Hal.	eur, as1,2, am1,2	Na	P	Abs	Synopsis Muscorum Frondosorum omnium hucusque Cognitorum 1: 356. 1848
33	*Dicranum dubium* Thér. & Dixon	oc	Nr	P	Nr	Revue Bryologique 48: 12. 1921
34	*Dicranum eggersii* Müll. Hal.	am3	Nr	Nr	Nr	Genera Muscorum Frondosorum 287. 1900
35	*Dicranum elongatum* Schleich. ex. Schwägr.	eur, as1,2, am1	No	M	Abs	Species Muscorum Frondosorum, Supplementum Primum 1: 171. pl. 43. 1811
36	*Dicranum filum* Bory	afr3	Nr	P	Nr	Voyage dans les Quatre Principales Îles des Mers d'Afrique 3: 17. 1804

37	*Dicranum flagellare* Hedw.	eur, as1,2, afr1, am1,2	No	M	Bfl	Species Muscorum Frondosorum 130. 1801
38	*Dicranum fragilifolium* Lindb.	eur, as1,2, am1	Na, No	M	B, Bfl	Botaniska Notiser 1857: 147. 1857
39	*Dicranum fragillimum* Warnst.	as3	Nr	Nr	Nr	Hedwigia 57: 78. 14. 1915
40	*Dicranum frigidum* Müll. Hal.	am2,4	Na	P	Abs	Botanische Zeitung (Berlin) 17: 219. 1859
41	*Dicranum fulvum* Hook.	eur, as2, am1	Nr	M	B	Musci Exotici 2: 149. 1819
42	*Dicranum fuscescens* Turner	eur, as1,2, am1,5	Nr	M	Abs	Muscologiae Hibernicae Spicilegium 60. pl. 5: f. 1. 1804
43	*Dicranum gonoi* Cardot	as2	Nr	Nr	Nr	Bulletin de la Société Botanique de Genève, Sér. 2 1: 121. 1909
44	*Dicranum gregoryi* B. H. Allen	am4	Nr	Nr	B	The Bryologist 91: 91. f. 1-7. 1988
45	*Dicranum groenlandicum* Brid.	eur, as1,2, am1	No	M	Abs	Muscologia Recentiorum Supplementum 4: 68. 1819
46	*Dicranum hamulosum* Mitt.	as2	No	M	Nr	Transactions of the Linnean Society of London, Botany 3: 156. 1891
47	*Dicranum himalayanum* Mitt.	as3	No	M	Abs	Journal of the Proceedings of the Linnean Society, Botany, Supplement 1: 14. 1859
48	**Dicranum homannii* Boeck	eur	Nr	Nr	Nr	Handbok i Skandinaviens Flora, Andra Upplagen 314. 1832
49	*Dicranum howelli* Renauld & Cardot	am1	Na	Nr	Nr	Revue Bryologique 15: 70. 1888
50	*Dicranum japonicum* Mitt.	as2	Na	M	Abs	Transactions of the Linnean Society of London, Botany 3: 155. 1891.
51	*Dicranum johnstonii* Mitt.	afr2	Na	M	Abs	Journal of the Linnean Society, Botany 22: 300. 1886.
52	*Dicranum kashmirense* Broth.	as3	No	M	Abs	Acta Societatis Scientiarum Fennicae 24 (2): 9. 1899
53	*Dicranum klauteri* Reimers	as2	Nr	Nr	Nr	Hedwigia 70: 363. f.1-4. 1931
54	**Dicranum kwangtungense* (P.C. Chen) T. J. Kop.	as2	Nr	Nr	Nr	Bryobrothera 1: 200. 1992
55	*Dicranum leiodontium* Cardot	as2	No	M	Nr	Bulletin de l'Herbier Boissier, sér. 2, 7: 714. 1907
56	*Dicranum leioneuron* Kindb.	am1	Na	M	Bfl	Bulletin of the Torrey Botanical Club 16: 92. 1889
57	*Dicranum leucobryoides* Besch. ex. Müll. Hal.	am6	Nr	Nr	Nr	Genera Muscorum Frondosorum 285. 1900

58	*Dicranum levieri* Müll. Hal.	eur	Nr	Nr	Nr	Genera Muscorum Frondosorum 285. 1900
59	*Dicranum linzianum* C. Gao	as2	No	Nr	Nr	Acta Phytotaxonomica Sinica 17: 115. 1979
60	*Dicranum longicylindricum* C. Gao & T. Cao	as2	Nr	Nr	Nr	Bryobrothera 1: 218. 1992
61	**Dicranum longipilum* Müll. Hal.	eur, afr2	Nr	Nr	Nr	Synopsis Muscorum Frondosorum omnium hucusque Cognitorum 1: 411. 1848
62	**Dicranum longirostratum* (P. Beauv.) Brid.	am1	Nr	Nr	Nr	Muscologia Recentiorum Supplementum 1: 228. 1806
63	*Dicranum lophoneuron* Müll. Hal.	am2	Nr	M	Nr	Synopsis Muscorum Frondosorum omnium hucusque Cognitorum 2: 589. 1851
64	*Dicranum lorifolium* Mitt.	as2,3	Na	M	Abs	Journal of the Proceedings of the Linnean Society, Botany, Supplement 1: 15. 1859
65	**Dicranum macrogaster* Müll. Hal.		Nr	M	Nr	Hedwigia 39: 252. 1900
66	*Dicranum majus* Turner	eur, as1,2, am1	Na	P	Abs	Muscologiae Hibernicae Spicilegium 59. 1804.
67	*Dicranum mayrii* Broth.	as2	Na	M	Bfl	Hedwigia 38: 207. 1899
68	*Dicranum montanum* Hedw.	eur, as1,2,3, am1	No	M	Bfl	Species Muscorum Frondosorum 143. pl. 35: f. 8-13. 1801
69	**Dicranum morenoi* Müll. Hal.	am6	Nr	Nr	Nr	Hedwigia 36: 97. 1897
70	*Dicranum muehlenbeckii* Bruch. & Schimp.	eur, as1,2, am1	Na	M, P	Abs	Bryologia Europaea 1: 142. 78 (fasc. 37-40 Mon. 38. 30). 1847.
71	**Dicranum myosuroides* DC.	eur	Nr	Nr	Nr	Flore Française. Troisième Édition 6: 222. 1815
72	*Dicranum nipponense* Besch.	as2	No	M	Abs	Annales des Sciences Naturelles; Botanique, sér. 7, 17: 332. 1893.
73	**Dicranum nitidum* (Dozy & Molk.) Dozy & Molk.	as2,3,4	No	M	Nr	Plantae Junghuhnianae 3: 330. 1854
74	**Dicranum novae-hollandiae* Turton	austr1	Nr	Nr	Nr	A General System of Nature 2: 1717. 1806
75	*Dicranum novaustrinum* Margad.	austr1	Nr	M	Nr	Lindbergia 1: 127. 1972
76	*Dicranum obliquatum* Mitt.	afr2	Nr	Nr	Nr	Journal of the Proceedings of the Linnean Society 7: 148. 1863
77	*Dicranum ontariense* W.L. Peterson	am1, eur	Na	M	Abs	Canadian Journal of Botany 55: 988. 1977
78	*Dicranum orthophylloides* Dixon	as3	Nr	Nr	Nr	Notes from the Royal Botanic Garden, Edinburgh 19: 280. 1938
79	*Dicranum orthophyllum* Broth.	as2	Nr	M	Nr	Symbolae Sinicae 4: 27. 1929

80	**Dicranum otii* (Sakurai) Sakurai	as2,3,4	Nr	Nr	Nr	Journal of Japanese Botany 27: 157. 1952
81	**Dicranum pachyneuron* (Molendo) Kindb.	eur	Nr	Nr	Nr	European and N. American Bryineae (Mosses) 2: 190. 1897
82	**Dicranum pacificum* Ignatova & Fedosov	as1	Nr	M	B	Arctoa, a Journal of Bryology 17: 76. 2008
83	**Dicranum pallescens* (Besch.) Müll. Hal.	afr3	Nr	Nr	Nr	Genera Muscorum Frondosorum 262. 1900
84	*Dicranum pallidisetum* (J. W. Bailey) Ireland	am1	No	M, P	Abs	The Bryologist 68: 447. 1965
85	*Dicranum papillidens* Broth.	as2	No	M	Abs	Akademie der Wissenschaften in Wien, Sitzungsberichte, Mathematisch-naturwissenschaftliche Klasse, Abteilung 1 133: 561. 1924
86	**Dicranum perichaetiale* (P. Beauv.) Brid.	afr3	Nr	Nr	Nr	Muscologia Recentiorum Supplementum 1: 204. 1806
87	*Dicranum peruvianum* H. Rob.	am4	Na	P	Abs	The Bryologist 70: 317. 1967
88	*Dicranum petrophylum* G. Negri	afr2	Nr	Nr	Nr	Annali di Botanica 7: 162. 1908
89	**Dicranum pinetorum* Griff.	as3	Nr	Nr	Nr	Calcutta Journal of Natural History and Miscellany of the Arts and Sciences in India 2: 497. 1842
90	*Dicranum polysetum* Sw.	eur, as1, am1,2	Na	P	Abs	Monthly Review 34: 538. 1801
91	*Dicranum psathyrum* Klazenga	as2,3,4	Na	M, P	Abs	Journal of the Hattori Botanical Laboratory 87: 118. 1999
92	**Dicranum pseudacutifolium* Otnyukova	as1	Nr	M	Nr	Arctoa, a Journal of Bryology 16: 163. 2007
93	*Dicranum pseudofalcatum* Seppelt	austr2	Nr	Nr	Abs	The Bryologist 83: 591. 1980
94	**Dicranum pseudojulaceum* (Müll. Hal.) Müll. Hal.	am5	Nr	Nr	Nr	Hedwigia 39: 259. 1900
95	*Dicranum pseudoleucoloma* Müll. Hal.	am6	Nr	Nr	Nr	Linnaea 43: 397. 1882
96	*Dicranum pseudorobustum* Müll. Hal. ex. Geh.	austr2	Nr	Nr	Nr	Revue Bryologique 4: 53. 1877
97	*Dicranum rectifolium* Müll. Hal.	as2	Nr	Nr	Nr	Nuovo Giornale Botanico Italiano, new series 3: 98. 1896
98	*Dicranum rhabdocarpum* Sull.	am1,2	No	M	Abs	Memoirs of the American Academy of Arts and Science, new series 4: 172. 3. 1849
99	**Dicranum richardsoni* Drumm.	am1	Nr	Nr	Nr	Musci Americani; or, Specimens of the Mosses Collected in British North America 104. 1828
100	*Dicranum rodriguezii* Müll.	afr3	Nr	Nr	Nr	Genera Muscorum

	Hal.					Frondosorum 285. 1900
101	*Dicranum savatieri* (Besch.) Schimp. ex. Paris	as2	Nr	Nr	Nr	Index Bryologicus, editio secunda 2: 57. 1904
102	**Dicranum saxatile* Lag., D. Garcia & Clemente	eur	Nr	M	Abs	Anales de Ciencias Naturales 5: 178. 1802
103	*Dicranum schensianum* Müll. Hal.	as2	Nr	Nr	Nr	Nuovo Giornale Botanico Italiano, new series 4: 249. 1897
104	*Dicranum scoparium* Hedw.	eur, as1,2,3,5, am1,2,4,afr1, austr2	Na, No	M, P	Abs	Species Muscorum Frondosorum 126. 1801
105	**Dicranum scopellifolium* Müll. Hal.	as2	Nr	M	Nr	Nuovo Giornale Botanico Italiano, new series 5: 169. 1898
106	*Dicranum scottianum* Turner ex Scott, Robert	eur, as1,2, am1	No	Nr	M	Transactions of the Dublin Society 3: 158. pl. 2. 1803.
107	**Dicranum scrabrophyllus* Müll. Hal.	am5	Nr	M	Nr	Hedwigia 36: 84-144. 1897
108	**Dicranum seligeri* Brid.	eur	Nr	Nr	Nr	Muscologia Recentiorum Supplementum 4: 59. 1819
109	*Dicranum semperi* Hampe	as4	Nr	Nr	Nr	Genera Muscorum Frondosorum 297. 1900
110	*Dicranum setifolium* Cardot	as2	No	M	Nr	Bulletin de l'Herbier Boissier, sér. 2, 7: 714. 1907
111	*Dicranum spadiceum* J.E. Zetterst	eur, as1, am1	Na	M, P	Abs	Kongliga Svenska Vetenskapsakademiens Handlingar 5(10): 20. 1865
112	*Dicranum speirophyllum* Mont.	oc	Nr	Nr	Nr	Annales des Sciences Naturelles; Botanique, sér. 2, 20: 295. 1843
113	*Dicranum splachnoides* Brid.	eur	Nr	Nr	Nr	Journal für die Botanik 1800: 295. 1801
114	*Dicranum spurium* Hedw.	eur, as1,3, am1	Na	M	Abs	Species Muscorum Frondosorum 141. 1801.
115	**Dicranum strictum* (Dicks.) Sm.	eur	Nr	Nr	Nr	Flora Britannica 3: 1218. 1804
116	**Dicranum stygium* Brid.	eur	Nr	Nr	Nr	Muscologia Recentiorum Supplementum 4: 64. 1819
117	**Dicranum sulphureo-flavus* Müll. Hal.	austr2	Nr	Nr	Nr	Index Bryologicus Supplementum Primum 98. 1900
118	*Dicranum sumichrastii* Duby	am2	Nr	P	Nr	Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 20: 353. 1870
119	*Dicranum symblepharoides* Cardot	as2	Nr	M	Nr	Bulletin de la Société Botanique de Genève, Sér. 2 1: 121. 1909
120	*Dicranum syrrhopodontoides* Müll. Hal.	am6	Nr	M	Nr	Hedwigia 36: 96. 1897

121	*Dicranum tauricum* Sapjegin	eur, afr4, am1	No	M	G, B	Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 46 (Beibl. 105): 10. pl. 2: f. 19. 1911
122	*Dicranum thelinotum* Müll. Hal.	as2	Nr	Nr	Nr	Nuovo Giornale Botanico Italiano, new series 3: 98. 1896
123	*Dicranum thraustophyllum* Müll. Hal.	as4	Nr	Nr	Nr	Genera Muscorum Frondosorum 297. 1900
124	*Dicranum toninii* Müll. Hal.	am6	Nr	M	Nr	Hedwigia 36: 97. 1897
125	**Dicranum torquatum* Mitt.	austr1,2	Nr	Nr	Nr	Journal of the Proceedings of the Linnean Society 4: 69. 1859
126	*Dicranum transsylvanicum* Luth	eur	Na	Nr	Abs	Cryptogamie Bryologie 23: 18. f. 1--2. 2002
127	**Dicranum truncatum* (Müll. Hal.) Müll. Hal.	am6	Nr	Nr	Nr	Synopsis Muscorum Frondosorum omnium hucusque Cognitorum 1: 410. 1848.
128	*Dicranum truncicola* Broth.	as2	Nr	Nr	Nr	Akademie der Wissenschaften in Wien, Sitzungsberichte, Mathematisch-naturwissenschaftliche Klasse, Abteilung 1 1, 133: 561. 1924
129	*Dicranum tubulifolium* Ireland	am4	Na	P	Abs	The Bryologist 87: 355. 1984
130	*Dicranum undulatum* Schrad. ex. Brid.	eur, as1,2,3, am1	Na	M	Abs	Journal für die Botanik 1800(2): 294. 1801
131	*Dicranum viride* (Sull. & Lesq.) Lindb.	eur, as1,2, am1	No	M	B	Hedwigia 2: 70. 1863
132	*Dicranum yezomontanum* Nog.	as2	Nr	Nr	B	Journal of the Hattori Botanical Laboratory 8: 18. 1952

Vingt-cinq pourcent des espèces de *Dicranum* présentent un dimorphisme sexuel qui se caractérise par des pieds mâles de taille très réduite, épiphytes des plantes femelles, que l'on appelle des mâles nains (Figure 7, Figure 8, **Tableau 2**). Leur origine, et leurs conséquences évolutives sont discutées dans l'article 3 du chapitre 3.

Figure 7: (A) Mâles nains sur un pied femelle de *D. undulatum*, (B) polysétie chez D. majus, (C) monosétie chez *D. scoparium*

Un autre groupe d'espèces possède des structures de reproduction asexuée plus ou moins spécialisées : gemmules, branches flagelliformes, feuilles avec une zone de cassure (Figure 8) ; leurs permettant de réaliser de la propagation végétative. Hedenäs & Bisang (2004) notent cependant que tous les *Dicranum* présents en Europe produisent des branches flagelliformes à des fréquences plus ou moins élevées. Reproduction végétative et reproduction sexuée peuvent avoir lieu simultanément ou non au cours de la vie des espèces. Les différents types de production de soie, la présence de dimorphisme sexuel, ainsi que la présence d'organes de reproduction végétative sont synthétisés dans le **Tableau 2**.

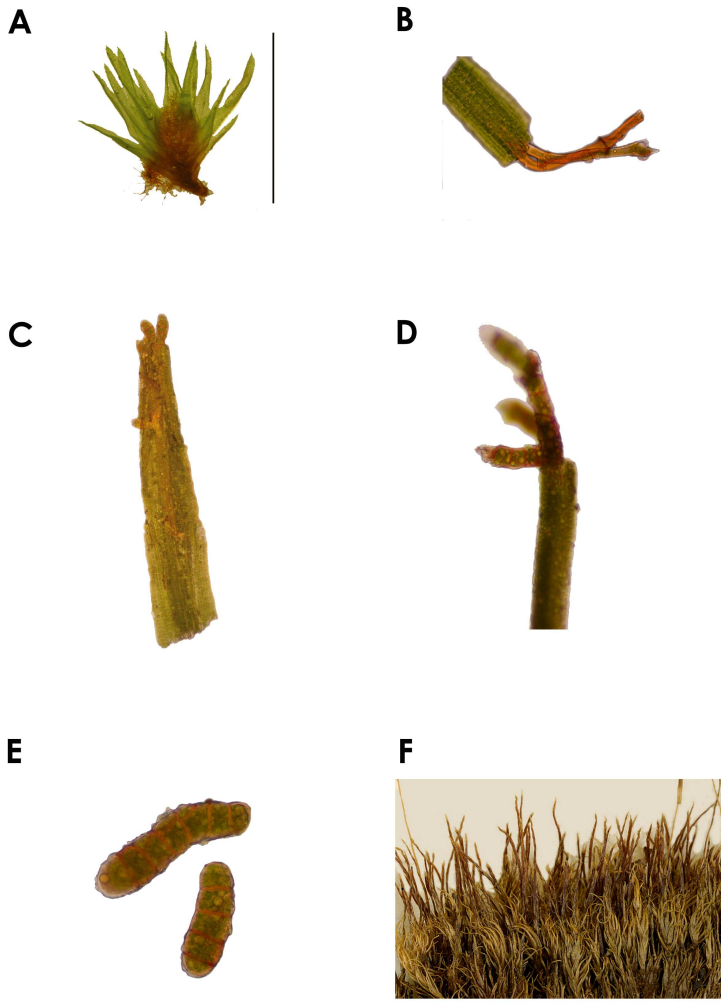

Figure 8: (A) Mâle nain chez *Dicranum undulatum* Schrad. ex Brid. (scale: 3 mm), (B) Apex de feuille cassé avec protonéma chez *D. viride* (Sull. & Lesq.) Lindb., (C - E) Gemmules chez *D. tauricum* Sapjegin, (F) Branches flagelliformes chez *D. flagellare* Hedw.

La majorité des espèces sont recensées dans l'hémisphère nord (Figure 9, Figure 10) et le centre de diversité est localisé en Asie (Chine, Japon, Mongolie, Corée et Taiwan, **Tableau 3**) avec 45 espèces dénombrées (**Tableau 2**). Plus de 20 espèces sont également inventoriées pour la Russie orientale, l'Amérique du nord et l'Europe. Globalement, le taux d'endémisme est relativement élevé puisque dans neuf des zones géographiques illustrées dans la Figure 10, plus de 50 % des espèces sont endémiques. Cependant, ce chiffre est à relativiser lorsque l'on sait que 70 % de ces espèces ont été décrites entre 1801 et 1900 sur la base de peu de caractères morphologiques. Encore actuellement 29 % espèces du genre *Dicranum* ne sont connues qu'à travers le spécimen type et la diagnose princeps associée (**Tableau 2**). Inversement certaines espèces telles que *D. majus* Tur. sont présentes sur l'ensemble de l'hémisphère nord et d'autres ont une répartition mondiale (ex. *D. scoparium*).

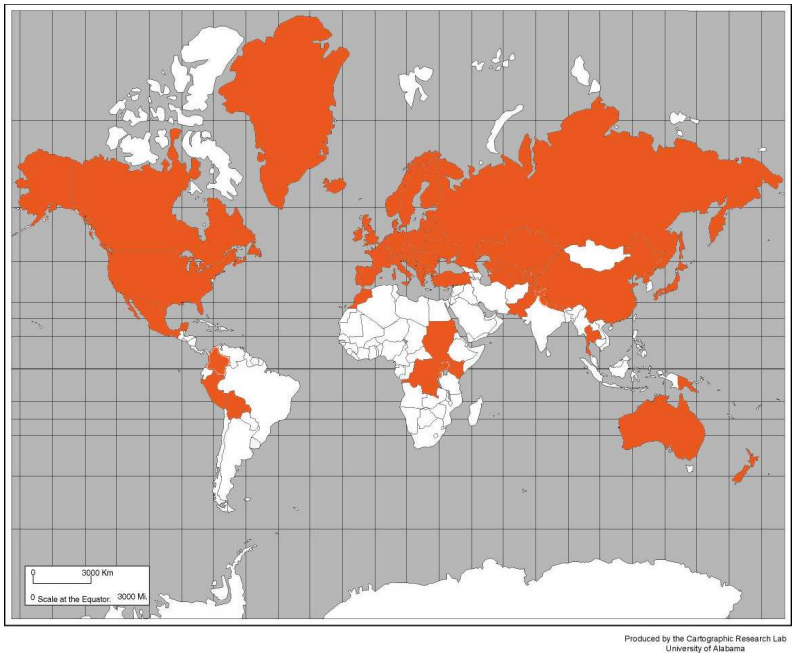

Figure 9 : Carte de distribution du genre *Dicranum*, Amérique, d'après: He, 1998 ; Worley & Iwatsuki, 1970 ; Thériot, 1932 ; Menzel, 1992 ; Bellolio-Trucco & Ireland, 1990; Florschütz-De Waard & Florschütz, 1979; Hermann, 1976; Golberg, 2003 ; Afrique, d'après : d'après **: Bizot, 1973 ; Demaret, 1940 ; Jelenc, 1949-1953 ; <u>Océanie,</u>** d'après **: Brown, 1896 ; Norris & Kopponen, 1990 ; <u>Asie,</u>** d'après **: Iwatsuki, 2004 ; Osada, 1958 ; Higishi & Nishimura, 2003 ; Noguchi, 1954 ; Redfearn & Wu, 1986 ; Gao & Cao, 1992 ; Mitten, 1859 ; Thériot ; 1918 ; Frey & Kürschner, 2009 ; Müller, 2009 ; El-Oqlah & al., 1988 ; He, 1996 ; Ninh, 1993 ; Lin, 1981 ; O'Shea, 2003 ; <u>Europe,</u>** d'après**: Colacino & Sabovljevic, 2006 ; Natcheva & Ganeva, 2005 ; Ignatov & Afonina, 1992 ; Hill & al, 2006 ; Casas & al., 2006** ; Bergþór, 2003

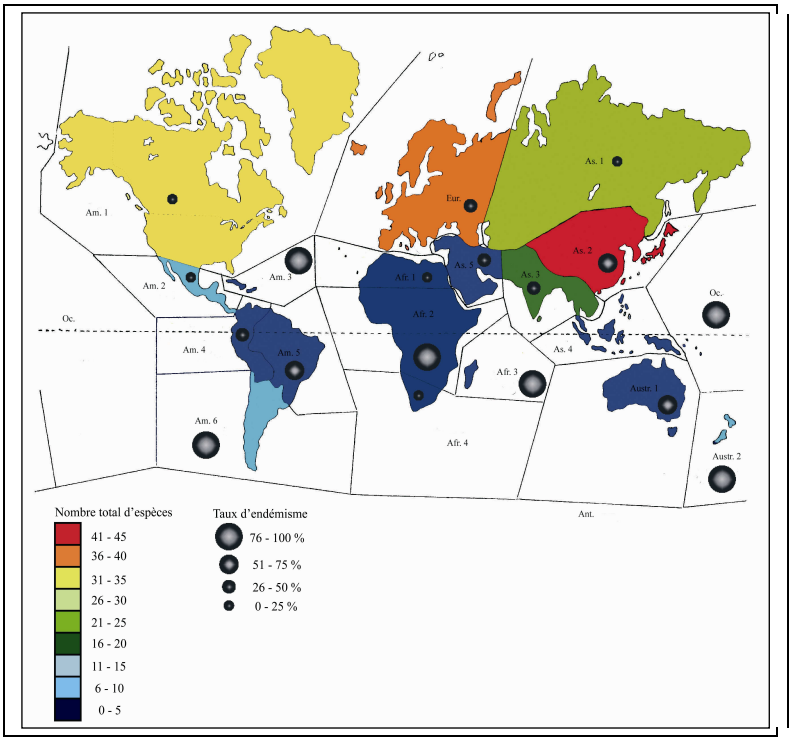

Figure 10: Répartition et taux d'endémisme du genre *Dicranum* par zones géographiques

Cependant un défaut de prospection entraînant un biais d'échantillonnage est notable pour certaines régions du monde (ex. Mongolie, Afrique du nord, montagnes du Cameroun) (Figure 1). Ainsi, aucun inventaire bryologique n'a été publié pour le Maghreb depuis Jelenc (1967). Le genre est connu pour être présent au Maroc (Jelenc, 1949-1953), mais n'est pas signalé en Algérie et en Tunisie ; or il est fort probable qu'on le retrouve au sein des contextes sylvatiques d'altitude plus ou moins arrosés de l'Atlas tellien. De même, des recherches en Mongolie, Irak, Iran ou tout simplement la révision des récoltes de ces régions, devraient souligner la présence d'espèces du genre dans ces pays. *Dicranum borbonicum* n'est connu qu'à travers deux récoltes anciennes (PC0029525, PC0029526) et aucune des prospections récentes réalisées sur l'île de la Réunion (com. pers. Bardat & Ah-Peng) n'ont permis d'en retrouver des populations.

En plus d'une répartition mondiale, le genre *Dicranum* possède une grande amplitude écologique avec des espèces croissant du niveau de la mer jusqu'à des altitudes de 4 500 m (Briggs, 1965). On les retrouve également sur des substrats variés : humus (ex. *D. majus*), bois pourrissants (ex. *D. tauricum*), troncs (ex. *D. viride*), et dans des écosystèmes variées : semi toundra (ex. *D. fuscescens*, Hicklento & Oechel, 1976), forêts, tourbières (ex. *D. bonjeanii*). Mais bien que certaines espèces croissent dans un grand nombre d'habitats (ex. *D. scoparium*, Briggs, 1965), d'autres espèces sont inféodées à des milieux spécifiques tels que les tourbières.

Tableau 3: Synthèse du nombre total d'espèces, nombre d'espèces endémiques, nombre d'espèces présentes dans l'hémisphère nord, nombre d'espèces cosmopolites par zones géographiques définies selon van der Wijk & *al. (*1962)

	As1	As2	As3	As4	As5	Am1	Am2	Am3	Am4	Am5	Am6	Eur	Af1	Af2	Af3	Af4	Ausrt1	Austr2	Oc
Nombre total d'espèces	23	45	17	4	2	32	8	1	6	4	8	38	1	5	5	1	4	6	2
Nombre d'espèces endémiques	3	26	8	1	1	8	2	1	4	3	8	14	0	4	5	0	3	5	2
Nombre d'espèces présentes dans l'hémisphère nord	19	14	4	-	-	21	3	-	-	1	-	21	1	-	-	-	-	-	-
Nombre d'espèces cosmopolites	1	1	1	-	1	2	1	-	1	-	-	2	-	-	-	1	-	-	-
Autres (non endémiques, ni cosmopolites)	-	4	4	3	-	1	2	-	1	-	-	1	-	1	-	-	1	1	-

Les relations de parentés au sein du genre *Dicranum* sont encore actuellement inconnues. Une étude phylogénétique récente (Lafarge & *al*., 2002) basée sur deux marqueurs chloroplastiques (*rbcL* et *rps4*) a montré la monophylie d'un groupe *Dicranum sensu lato* composé des genres *Dicranum*, *Paraleucobryum*, *Chorisodontium* et *Orthodicranum* ; groupe frère d'un clade composé des genres *Holomitrium* et *Eucamptodontopsis*. Cependant, les relations phylogénétiques au sein de *Dicranum sensu lato* ne sont pas résolues.

Ce chapitre présente la première étude moléculaire centrée sur le genre *Dicranum*. (Article 1).

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Article 1: Pichonet, A., Hassanin, A., Couloux, C., &. Bardat, J. Molecular phylogeny and DNA barcoding of *Dicranum* s.l. (Dicranaceae, Bryopsida). To be submitted in Molecular Phylogenetics and Evolution

to be submitted to Molecular Phylogenetics and Evolution

Molecular phylogeny and DNA barcoding of *Dicranum* s.l. (Dicranaceae, Bryopsida)

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Abstract

The phylogeny and taxonomy of *Dicranum sensu lato* using 243 specimens collected from 26 *Dicranum* species all over the world, and closely-related genera *Paraleucobryum* (*P. longifolium*, *P. enerve*), and *Pseudochorisodontium* (*P. gymnostomum*, *P. hokkinense*) was studied using four markers from the three genomes. The relevance of the morphological characters traditionally used in the classifications (flagelliform branches, caducous leaves, gemmae, lack of teeth peristome) and life cycle traits (male dwarfism) was studied in the light of our phylogenetic results. The monophyly of Dicranoideae was strongly supported, and *Dicranum* s.l. (*Dicranum*, *Paraleucobryum*, *Pseudochorisodontium*) was found to be monophyletic, sister group to genus *Holomitrium.* Eleven species were found monophyletic, but six polyphyletic species were revealed, leading to the discovery of three cryptic species. The type species *D. scoparium* is not monophyletic and was found to occur in Sub-Saharian Africa.

Keywords barcode; Bryopsida; *Dicranum*; *Gap*C; molecular phylogeny; *Nad*5-4; *rbc*L; *rpl*32-*trn*L

1. **Introduction**

The genus *Dicranum* Hedw. (Greek *dicranon* = pitchfork) is one of the 41 genera of *Dicranaceae* Schimp. (Goffinet & *al*, 2008). It was established by Hedwig in 1801 for a group of mosses with a single ring of bifid peristome teeth, dioicous sex distribution and terminal androecia. Hedwig recognized 34 species in the genus, among them, five are still retained including the type species, *D. scoparium* Hedw..

Dicranum with over 885 published binomials (van der Wijk & *al.*, 1962; Crosby & *al*., 1990; www.tropicos.org) and an estimated number of 130 species (Pichonet, submitted) is characterized by 1) the straight to homomallous to falcate-secund leaves with a rather narrow costa (<1/3 of leaf width), 2) the lamina cells which are smooth or mammillose, and often porose, 3) the alar cells always enlarged, usually bistratose, 4) costa often dorsally ridged, 5) perichaetia often polysetous, and 6) capsules erect or inclined, striate or plicate when dry (Crum & Anderson, 1981). Twenty-five percent of the species are known only from the type specimen (Pichonet, submitted). The genus is mainly distributed in the Northern Hemisphere, and the highest species diversity is described in eastern Asia (Takaki, 1972). The species grow in loose to dense mats on various, horizontal to vertical, substrates from sea level to up to 4500 m (Briggs, 1965). All species in the genus *Dicranum* are dioicous, and a peculiar reproductive feature is the occurrence of male dwarfism (small, epiphytic male stems), which is found in 25% of the species (Pichonet, submitted). Moreover, many species can reproduce asexually by caducous leaf apices or gemmae and flagelliform branches occur with different frequencies in all *Dicranum.*

The lack of knowledge of the variability of most of the species, the use of variable characters states, the mostly regional revisions and studies (Briggs, 1965; Bellolio-Trucco & Ireland, 1990; Takaki, 1964, 1972; Gao & Cao, 1992), and the lack of a worldwide revision, made this genus a difficult one in species identification and species delineation. Its numerous species are mostly recognized based on variation in leaf shape and size, cells shape and size, and costa structure. But, *Dicranum* is very variable in size and habit, especially leaf shape may vary considerably within one species (e.g. *D. scoparium*) giving rise to plants with a completely different appearance within the same species. For example, Moss Flora of North America mentioned *D. scoparium* as “undoubtedly the most polymorphic species of the genus in North America” with “the habit of the leaf shape varying from the typical lanceolate and long-acuminate to the odd ovate-lanceolate and short-acuminate”(see also Briggs, 1965). Furthermore, identifications at species level can be complicated in some cases when the

morphological characters overlapped (e.g. number of lamellae in the upper part of the costa) (e.g. *D. muehlenbeckii* is commonly confused with *D. brevifolium* and *D. spadiceum;* or *D. laevidens* confused with *D. angustum*, Dierssen, 2001). In such cases, the DNA barcoding approach represents an expedient tool for species identification. The standard plant barcode is based on the combination of a portion of two chloroplastic coding genes, *rbc*L and *mat*K (CBOL Plant Working Group, 2009). Although *rbc*L is commonly used in bryophytes, the PCR amplification of *mat*K is problematic (Cräutlein & *al.* 2011).

The phylogeny of *Dicranum* is still largely unknown. Using two chloroplast markers, Lafarge & *al.* (2002) have concluded that the subfamily Dicranoideae is monophyletic, and that it is composed of two groups: the first one, and here named *Dicranum sensu lato,* contains species classified in *Dicranum* (*D. majus, D. scoparium, D. muehlenbecki, D. condensatum, D. fulvum,* and *D. flagellare*) but also *Chorisodontium* (*C. mittenii*, and *C. setaceum*) and *Paraleucobryum* (*P. longifolium*), and the other represented by *Holomitrium* (*H. borbonicum*, and *H. cylindraceum*), and *Eucamptodon* (*E. muelleri*) Furthermore, molecular evidence has shown that the genus *Dicranoloma* does not belong in *Dicranum sensu lato* (Stech, 1999; Lafarge & *al*., 2002; Hedderson & *al.*, 2004; Stech & *al.*, 2006), in contradiction with the morphological cladistic analyses of Norris & Koponen (1990) and Klazenga (1999). Therefore, all previous molecular studies have revealed high levels of homoplasy in the morphological characters used for both phylogeny and taxonomic identification keys.

In the present study, we assess the capacity of one of the two plant barcode genes (*rbc*L), one additional chloroplastic marker (*rpl*32-*trn*L), two nuclear markers (ITS1, *Gap*C), and one mitochondrial marker (*Nad*5-4) to resolve the phylogeny and taxonomy of *Dicranum sensu lato* using 243 specimens collected from 26 *Dicranum* species all over the world, and closely-related genera *Paraleucobryum* (*P. longifolium, P. enerve*), and *Pseudochorisodontium* (*P. gymnostomum, P. hokkinense*). A further goal is to evaluate the relevance of the morphological characters traditionally used in the classifications (flagelliform branches, caducous leaves, gemmae, lack of teeth peristome) and life cycle traits (male dwarfism) in the light of our phylogenetic results.

2. Material and methods

2.1 Taxon sampling and species identification

Trying to resolve the phylogenetic structure of a group of living organisms (e.g., attesting the molecular monophyly or non-monophyly) implies practical constraints on data collection. Indeed, the likelihood of detecting polyphyly is directly linked to the sampling strategy. When interspecifically shared alleles causing polyphyly are rare, very intensive sampling may be required to document this pattern (Wiens & Servedio, 2000 *in* Funk & Omland, 2003). As advised by Funk & Omland (2003), we tried to include all species believed *a priori* to be closely related, to maximize the geographic diversity of samples because of the potential for phylogenetic/cryptic species. In addition, we focused our sampling on areas of sympatry between studied species and important sources of biological variation where known (subspecies, ecotypes, morphological variants, etc.). A minimum of one individual per species for a total of 37 species of *Dicranum* sensu lato were included in the phylogenetic analysis. In agreement with the classification of Hill & *al.* (2006), species of *Orthodicranum* were considered as belonging to the genus *Dicranum*. Potential out groups were selected on the basis of the molecular phylogeny of the dicranoids (Dicranoideae) of Lafarge & *al.* (2002), and included *Pseudochorisodontium*, *Holomitrium*, *Dicranodontium*, and *Campylopus.* Only the dicranoid genus *Chorisodontium* was lacking from the data set.

Species identification is problematic for groups of organisms such as *Dicranum* that have not been treated systematically. A comprehensive monograph of the genus is lacking and identification was done using various regional revisions and local Flora's (NORTH AMERICA AND EUROPE: Bellolio-Trucco & Ireland, 1990; Crum & Anderson, 1981; Hedenäs & Bisang, 2004; Pedrotti, 2001; Smith, 2004; Hallingbäck & *al.*, 2006; ASIA: Gao & *al.*, 1999). Some samples were initially identified by local bryologists (Russia: M. Ignatov; USA: S. Schuette and B. Goffinet; The Netherlands: S. Laegaard; Sweden: L. Hedenäs), and double-checked by the authors. Nomenclature follows the latest taxonomic works, when available.

The occurrence of selected reproductive morphological characters traditionally used in classification (flagelliform branches, caducous leave, gemmae, presence/absence of peristome teeth, male dwarfism) were mapped on the species tree obtained from our molecular analysis.

2.2 Genomic sampling and alignment

2.2.1 Marker choice and primer design

As recommended by the CBOL Plant Working group (2009), a combination of genomic regions including barcode standards was analysed. Eight chloroplast regions (*rbc*L, *rpl*32-*trn*L$^{(UGA)}$, *atp*F-*atp*H, *psb*K-*psb*L, *psb*A-*trn*H, *mat*K, *rpo*C1, and *rpo*B), one mitochondrial region (*Nad*5-4) and two nuclear regions (ITS1, *Gap*C exon 6-exon10) were selected for molecular characters. Primers for amplifying and sequencing the ITS region (ITS5-bryo and ITSC-bryo) were based on Sabovljevic & *al.* (2005). Primers for amplifying and sequencing the *rbc*L region (F1_F and 2PRN_R) were based on Ebihara & *al*. (2007), primers for amplifying *atp*F-*atp*H, *psb*K-*psb*L, *psb*A-*trn*H, *mat*K, *rpo*C1, and *rpo*B were based on Kew barcoding (http://www.kew.org/barcoding/protocols.html), and primers for amplifying and sequencing the *rpl*32-*trn*L$^{(UGA)}$ region (*rpl*32 and *trn*L) were based on Shaw & *al*. (2007). Internal primers were designed for *rbc*L and *rpl*32-*trn*L$^{(UGA)}$ with respect to bryophytes. Primers for amplifying and sequencing the *Gap*C and *Nad* 5-4 regions were based on Pichonet & *al.* (in prep.). The primers are summarized in Table 3.

2.2.2 DNA extraction and PCR conditions

Plant DNA from distal portions of stems was extracted from fresh material using Quiagen Plant DNA Kit. Each sample was ground with two steel balls in a 2 mL tube for 2 min 30 s at 25 Hz in (Retsch Mixer Mill 300) .The buffer AP1 is modified by mixing (for 24 samples): 12 mL AP1; 0. 005 g BSA; 1,64 g of sucrose; 0. 48 g of SDS, and 24 µL of betamercaptoethanol. The step 1 was modified as following for the herbarium specimens: stems were lysed with 400 µL of AP1, 30 µL of CTAB and 30 µL of proteinase K for 24 hours at 42°C.

The standard PCR amplification conditions were as follows: (*rbc*L) initial denaturation at 94°C for 1 min; 40 cycles of denaturation at 94°C for 30 s, anneling at 49°C for 40 s, extension at 72°C for 40 s; final extension at 72°C for 5 min. (*rpl*32-*trn*L, see Shaw & *al.*, 2007), (*atp*F-*atp*H, *psb*K-*psb*L, *psb*A-*trn*H, *mat*K, *rpo*C1, and *rpo*B, see http://www.kew.org/barcoding/protocols.html) (*Gap*C) initial denaturation at 94°C for 1 min; 10 cycles of denaturation at 94°C for 30 s, anneling at 65°C for 1 min, extension at 72°C for 1 min, 35 cycles of denaturation at 94°C for 30 s, anneling at 60°C for 1 min, extension at 72°C for 1 min; final extension at 72°C for 5 min.

(ITS 1) initial denaturation at 94°C for 1 min; 10 cycles of denaturation at 94°C for 30 s, anneling at 55°C for 1 min, extension at 72°C for 1 min, 30 cycles of denaturation at 94 °C

for 30 s, anneling at 50°C for 1 min, extension at 72°C for 1 min; final extension at 72°C for 5 min. (*Nad*5-4) initial denaturation at 94°C for 1 min; 40 cycles of denaturation at 94°C for 30 s, anneling at 60°C for 1 min, extension at 72°C for 1 min; final extension at 72°C for 5 min.

PCR were purified using Quiagen kit and both strands were sequenced on ABI 3130. Thermocycler settings for the sequencing PCR were as follows: initial denaturation at 96°C for 1 min; 25 cycles of denaturation at 96°C for 10 s, anneling at 50°C for 5 s, extension at 60°C for 4 min. Some specimens were also sequenced by Genoscope.

Contigs were constructed from single-stranded forward and reverse sequences using Sequencher 3.0 (Gene Codes Corp.) and CodonCode Aligner (Codon Code Corp.). Sequences were aligned manually on SeAl v2.0a11 (Rambaut, 2002) and double checked by eye. Gaps were inserted where necessary to preserve positional homology.

2.2.3 Final data set used for phylogenetic construction

For this study, 243 specimens from 37 species were extracted and tested for PCR amplification. A high success was obtained for PCR amplification of *rbc*L (99%), *Gap*C (99%), *rpl*32-*trn*L (97%), and *Nad*5-4 (96%). However, we failed to amplify 12% of the ITS1 samples, including key taxa, such as *D. viride* and *D. fulvum*. In addition, the alignments of ITS1 sequences were problematic and outgroup comparisons were not possible. Therefore, ITS1 was not used for phylogenetic reconstruction. As a result, 225 specimens from 30 species were available for analysis of four markers.

For analytical convenience (less taxa), all individuals from a single country displaying identical sequences across the four loci were assigned to one haplotype. A total of 143 haplotypes were included in the final matrix.

2.3 Phylogenetic analysis

Separate and combined phylogenetic analysis of DNA sequences were carried out using maximum likelihood (ML) and Bayesian inference (BI) in which sequence insertions and deletions were coded.

The ML analysis were conducted using the GTR-CAT model, as it requires significantly reduced memory consumption and yields significantly better values than the widely used GTR+I+G likelihood model (Stamatakis, 2006) with RAxML v.6 (Stamatakis & *al.*, 2005). Branch support was assessed by bootstrapping (Felsenstein, 1985).

The Bayesian approach was performed using MrBayes 3.1.2 (Ronquist & *al.*, 2003), with the GTR+I+G model, four chains of 10,000,000 iterations, with trees sampled every 100

generations. The number of generations needed to reach stationary in the Markov chain Monte Carlo algorithm was estimated by visual inspection of the plot of the BI score at each sampling point. The first 50,000 trees for each run were excluded from the tree set ('burn-in'), and the remaining trees were combined to form the full sample of trees assumed to be representative of the posterior probability distribution.

2.4 Supermatrix versus SuperTRI approach

Supermatrix method implicitly assumes that all characters have experienced the same branching history. However, this assumption is not always valid, and conflicting phylogenetic signals between data sets may result in robust topological incongruence that can be misleading for understanding the real evolutionary history of taxa (Ropiquet & *al.*, 2009). Contrary to the supermatrix method defined by the direct, simultaneous use of all the character evidence from all included taxa (Queiroz & Gatesy, 2006), in the superTRI approach, a supertree is constructed by using the branch support values (BP or PP) of all phylogenetic hypotheses produced during the bootstrap or Bayesian analysis of the independent data sets (Ropiquet & *al*., 2009). Exploiting the advantages of branch support analysis and reproductibility criterion to evidence the most reliable phylogenetic hypothesis, the superTRI method can be interpreted as a consensus tree of all branch support analysis. Moreover, superTRI approach shows less sensitivity to the methods of tree reconstruction, and is more accurate to interpret the relationships among taxa (Ropiquet & *al*., 2009).

3. Results

3.1 Parsimony informative sites

The final data set contains four markers, and represents an alignment of 2805 characters. Among them, 362 were found to be parsimony informative, representing 13% of the total alignment. The markers containing the higher percentages of parsimony informative sites are *Gap*C (20%) and *rpl*32-*trn*L (19%). The smallest percentages of informative characters were found for *rbc*L (9%) and *Nad*5-4 (7%) (Table 1).

3.2. Phylogeny of Dicranum s.l. and species delimitation

3.2.1 Tree topology

Five data matrices were analyzed using ML and BI methods of tree reconstruction: the four molecular markers (*rbc*L, *rpl*32-*trn*L, *Gap*C, and *Nad*5-4), and the matrix combining the four markers (supermatrix approach). The four markers were analyzed separately to evaluate

their own signal, and to detect potential cases of serious incongruence (strong contradicting node support) by comparing the topologies and nodal support under BI and ML methods. Under ML method, nodes supported by BP $\geq$ 50 were considered as reliable, whereas under BI method, nodes supported by PP $\geq$ 0.75 were considered as reliable. The results are summarized in Figure 2 and Figure 3, respectively for ML and BI methods.

After analyzing, four categories of node robustness were defined. The first category of "very reliable" nodes were supported by ML supermatrix and ML superTRI approaches, and were also found using BI supermatrix and BI superTRI approaches, and confirmed by two markers from different genomes in separate analysis; this represents 23% of the nodes, based on the ML topology. The second category of "reliable" nodes were supported by ML supermatrix and ML superTRI approaches, and were also found using BI supermatrix and BI superTRI approaches, and confirmed by one marker in separate analysis; this represents 19% of the nodes. The third category of "no reliable" nodes were supported either by the ML supermatrix and ML superTRI approaches or using BI supermatrix and BI superTRI approaches, and confirmed by one marker, but contradicted by another in separate analysis or nor confirmed by any of the separate analysis; this represents 51% of the nodes. The fourth category of "strongly unreliable" nodes was based on a strong contradiction between supermatrix and superTRI approaches; this represents 7% of the nodes. These resulting four categories were summarized and illustrated on the BI phylogram in Figure 4.

The tree reconstruction revealed topological incongruence between supermatrix and superTRI methods which is revealed by separated analysis. Thus, the ML analysis of separate data sets showed topological incongruence with the ML supermatrix analysis ranging from 17% to 8%. We find 17% of robust conflicts between the supermatrix and *Gap*C, 14% of robust conflicts between the supermatrix and *rpl*32-*trn*L, 12% of robust conflicts between the supermatrix and *rbc*L, and 8% of robust conflicts between the supermatrix and *Nad*5-4.

The BI analysis of separate data sets shows topological incongruence with the BI supermatrix analysis ranging from 12% to 5% of nodes. We find 12% of robust conflicts between the supermatrix and *rbc*L, 8% of robust conflicts between the supermatrix and *Gap*C, 8% of robust conflicts between the supermatrix and *rpl*32-*trn*L, and 5% of robust conflicts between the supermatrix and *Nad*5-4.

3.2.2 Taxonomy

The monophyly of Dicranoideae was strongly supported by both phylogenetic methods (BP = 100, PP = 1), in the combined and the separate analysis (Fig. 2 and Fig.3). *Dicranum* s.l. was found to be monophyletic, and sister group to genus *Holomitrium*, using the BI approach (PP = 1) but its monophyly lacked support in the ML analysis (BP = 49). The genus *Pseudochorisodontium* was not monophyletic, *P. gymnostomum* being nested inside *D. scoparium sensu lato* while *P. hokinense* being nested in *D. assanicum*. Moreover, the former genus *Orthodicranum* was found to be paraphyletic based on the very different position of *D. setifolium* being member of this group.

At the species level, the present study confirmed the monophyly of eleven species of *Dicranum sensu lato*, and highlights the existence of three putative cryptic species in Asia (Asian *D. tauricum, D. viride* and *D. montanum*). The African endemic species *D. johnstonii* was nested in a robust clade (BP = 100, PP = 1) composed of European and Canadian *D. scoparium* specimens, making the latter species paraphyletic. The morphological identification of a least four Asian (n° 809, 811, 726, and 822) and one Canadian (n° 497) specimens remained uncertain even knowing their phylogenetic position.

Finally, sister-group relationships were recovered for *Dicranum ontariense* and *D. undulatum*, *D. brevifolium* and *D. acutifolium*; *D. flexicaule* and *D. fuscescens*, *D. fulvum* and *D. viride*, *D. flagellare* and *D. montanum*. The backbone of *Dicranum* s.l. remained largely unresolved.

3.3 Evolution of some reproductive traits

The distribution of the reproductive traits mapped on the species tree of *Dicranum* s.l. is shown in Figure 3. The parsimony reconstruction reveal that the investigated characters are extremely homoplastic: caducous leaves appeared six times, flagelliform branches appeared two times, gemmae two times, lack of peristome teeth appeared two times, and dwarf males appeared four times.

4. Discussion

4.1 Choice of the markers as barcode

4.1.1 Comparison of the five markers examined

*Mat*K, ITS1, and five other chloroplastic markers (*rpo*B, *rpo*C1, *atp*F-*atp*H, *psb*K-*psb*L, and *psb*A-*trn*H) were tested for PCR amplification using various conditions on 20 *Dicranum* specimens (data not shown). The difficulties encountered for their amplification,

using available primers (Kress & *al*., 2005) forced us not to select them, in the present study. More specifically, these results also confirm the problems associated with the use of MatK as a barcode standard in cryptogams (bryophytes and ferns, CBOL Plant working group (2009); Stech & Quandt, 2010).

Of the four markers analysed to resolve the phylogenetic relationships within *Dicranum s.l.*, *rpl*32-*trn*L is new for bryophytes (no bryophyte accession in NCBI), whereas *Gap*C, and *Nad*5-4 are new for Dicranaceae. Their number of parsimony informative characters varies from 20% (*Gap*C) to 7% (*Nad*5-4).

From the plastid genome, *rbc*L, which has been traditionally used in bryophyte phylogenies, is still one of the five most frequently used regions, but it has only been employed at the family level and above in recent publications, due to its conserved nature (Stech & Quandt, 2010). The authors argued that this marker is not suitable for species and population level analysis or barcoding approaches in bryophytes, contrasting with Liu *& al.* (2010) and our results showing that rbcL alone strongly discriminate 40% of the nodes at the interspecific level (Fig. 2 and Fig.3). Furthermore, *rpl*32-*trn*L was not listed in the recommendations of the CBOL Plant Working Group (2009) as a standard plant barcode or supplementary locus, but it provided one the highest value of parsimonious sites (19%) in this study. Considered as one of the regions offering levels of variability previously unseen in the chloroplast genome of angiosperm (Shaw & *al.*, 2007), this locus possessed several desirable characteristics of putative barcodes such as universality, amplification success and straightforward alignment.

The mitochondrion, *Nad*5-4 spacer was found to perform poorly at the boundary between species (7% of parsimonious sites) confirming the lower variability of the mitochondrial genome compared to the plastid genome in bryophytes (Knoop, 2004). However, in a recent review of the current state of markers used in bryophytes, Stech & Quandt (2010) suggest that within the *nad* genes which are particularly rich in positionally stable introns (Knoop & Brennicke, 2002), the spacer *nad*5-*nad*4 IGS might be a promising molecular marker at higher taxonomic level, as underscored by the study of Wahrmund & *al*. (2009).

From the complex, biparental inherited nuclear genome, the multigene marker *Gap*C showed great variability in the number of parcimony-informative sites (20%). Up to date, using bryophyte model, *Gap*C has only been used for *Mitthyrridium* (Wall 2002, 2005), Calymperaceae (Fisher & al., 2007) and *Sphagnum* (Szövényi & *al*. 2006, 2007, 2009) in phylogenetic and phylogeographic contexts, respectively. Its utility for bryophyte

phylogenetic inference is less known, but in *Sphagnum*, GapC has low intraspecific variation, similar to ITS (Stech & Quandt, 2010). We show here the usefulness of GapC for infrageneric phylogenies especially in *Dicranum.*

At the moment, no universal stand-alone DNA barcoding marker for bryophytes is available, and it is unlikely that such a marker will be found (Stech & Quandt, 2010). However, in the present study, we show that the two new markers for *Dicranaceae*, i.e., *rpl*32-*trn*L and *Gap*C, satisfied the Barcode standards (good amplification success and high interspecific variability (>95%), and seem promising for phylogenetic studies.

4.1.2 Species resolution

Few studies focused on evaluating barcode markers in bryophytes whereas seed plants have received far more attention (e.g. Kress & *al.*, 2005; Chase & *al.*, 2007). Among the five published papers, only two of them focused on bryophytes (Kress & Erickson, 2007; Fazekas & *al.*, 2009; Hollingsworth & *al.*, 2009 but Liu & *al.*, 2010, 2011). While studying eight potential barcodes suggested for land plants (*atp*F-*atp*H, ITS2, *mat*K, *psb*K-*psb*I, *rbc*L, *rpo*B, *rpo*C1, *trn*H-*psb*A), and two popular phylogenetic markers (*trn*L-*trn*F and *rps4*) at high taxonomic level (family level), Liu & *al.* (2010) did not find any single region exhibiting clear discontinuity between inter- and intra-specific divergence like cox1 in animals; but *rbc*L has the highest significance of divergence and distinguish nearly 90% of the tested taxa. They concluded that *rbc*L, *rpo*C1, *rps*4, *trn*H-*psb*A and *trn*L-*trn*F are potential candidate regions for DNA barcoding in mosses.

Testing plant DNA barcoding loci at the species level involved extensive taxonomic sampling but seldom taken intra-specific variation into account. Using the standard plant barcode (*rbc*L + *mat*K), 70% of species are resolved (CBOL Plant Working Group, 2009). However, the species-dicriminatory power can greatly vary between taxa in bryophytes: from 53% in Grimmiaceae to 89.5% in Aytoniaceae for *rbc*L (Liu & *al.* 2011).

In the present study, we assess the discriminatory power of potential barcode markers at the species limits in mosses, using the genus *Dicranum*. It is worth noting that DNA barcoding loci is challenged when closely related species and populations from different geographical areas of the same species are considered (Liu & *al.*, 2011). In fact, ambiguous species circumscription, hybridization, polyploidy, incomplete sorting of ancestral polymorphism could led to a failure of species discriminatory. However, applying DNA barcoding to morphologically-based classifications help to resolve morphologically into natural groups leading to the discovery of cryptic species (Shaw, 2001) or avoiding

misidentification caused by phenotypic plasticity (e.g. Liu & *al*., 2011).

Here, the combination of four markers originating for the three genomes of the plant enable us to discriminate the morphological-based species belonging to genus *Dicranum*, resolving the monophyly of eleven species, discovering three cryptic species, and questioning the species concept and species circumscription of six other species. The species-dicriminatory power of this maker combination seems promising for phylogenetic studies considering the taxonomic difficulties of the studied genus.

4.2 Reliability of the results

In an attempt to resolve the interspecific *Dicranum* relationships, we analysed the selected markers both separately and in a combined data set using ML and BI which have the advantage of allowing the indel encoding compared to ML. The analysis conducted under supermatrix and superTRI methods allowed us to validate the observed topology using two methods; but more fundamentally, to detect robust topological incongruence in the supermatrix signal that could have been mislead for the understanding the real evolutioary history of taxon. Thus, the relationships within *D. scoparium sensu stricto* (Fig.4) are far more complicated than the signal of the supermatrix. Errors in phylogenetic reconstruction caused by the use of inappropriate methods or models, sequencing and aligment errors, taxonomic misidentifications, DNA contamination by other organisms, endogenous contamination, strong selection pressure, horizontal transfert events, and incomplete lineage sorting can explain the observed robust incongruent topology confirmed by the separated analysis. In the current study, we discount taxonomic misidentifications as a primary source of error as the specimen identity was confirmed by a second identification. Similarly, alignment cannot be an issue, as there are only a few short indels in *Gap*C and *rpl*32-*trn*L across *Dicranum* that are not subject to alignment ambiguity. However, according to the signal from the separate analysis (Fig.2 and Fig.3), DNA contamination for *Gap*C must be considered as a possible explanation for incongruence in this specific case, especially since this marker is known to belong to a multigenic family (e.g. Stone, 2006).

4.3 Phylogeny of Dicranum

4.3.1 Species concepts and species limits

Species concepts are models of the patterns brought about by the way the evolutionary process works under various conditions attempting to explain how phenetic variation is compartmentalized (Winston, 1999). Numerous models have been proposed that can be

grouped into five main headings: morphological (i.e. phenetic), biological, phylogenetic, ecological, and cohesive, leading to different species limits. In bryophyte studies, most descriptions conform to what can be regarded as the morphological or typological species concept (Szweykowski, 1984). Molecular techniques have transformed the ability of scientists to describe and define biological diversity, and determine species limits. While investigating the species delimitation using molecular data, many authors apply the phylogenetic or genealogical species concept that defines species in terms of monophyly (e.g., single lineage of an ancestor-descendant population) (Bickford & *al.,* 2006). The molecular data may turn out to be congruent with the morphological data, resolving the species as monophyletic, or not; in the latter case the species is non-monophyletic or the data are erroneous.

Polyphyly (e.g., non-monophyly) in the broad sense referred to both paraphyly (all the haplotypes of one or more species are phylogenetically nested within the haplotypes of a second, paraphyletic species), and narrow-sense polyphyly (various haplotypes from the polyphyletic species are phylogenetically interspersed with those of other species such that they are not phylogenetically contiguous with each other on the gene tree) (Funck & Omland, 2003). In a survey of 584 studies, Funck & Omland (2003) found that 44% of the 526 studied genera included at least one polyphyletic species. They concluded that polyphyly is a much more important phenomenon than is generally recognized. Non-monophyly can originate from several sources: inadequate phylogenetic information, excessive splitting or lumping of species (misidentifying intraspecific variation as species-level variation, and vice-versa) (e.g., Fernandez & *al*., 2006; Bickford & *al.,* 2006; Hentschel & *al*., 2007), interspecific hydridization, incomplete lineage sorting, and unrecognized paralogy.

Molecular study of the relationships within *Dicranum* s. l. has revealed the existence of six polyphyletic species among taxa for which at least two samples were sequenced, leading to the discovery of three cryptic species. Thus, *D. montanum, D. viride,* and *D. tauricum* collected in Asia are divergent from European and North American populations. Asian *D. viride* could belong to *D. hakkodense* which has recently been resurrected by Ignatova & Fedosov (2008), but type specimen has to be studied before any nomenclatural changes. However, in *D. tauricum* and *D. montanum*, we refrain from any nomenclatural changes at this stage as we were not able to find any morphological characters discriminating both groups.

Excessive splitting and lumping of species can explained the apparent polyphyly of the worldwide distributed species *D. scoparium*, including African and Asian endemic taxa. Thus, *Dicranum johnstonii*, one of the five endemic species of *Dicranum* from sub-Saharan

Africa (*D. acanthoneurum, D. borbonicum, D. obliquatum*, and *D. petrophyllum*, *in* O'Shea, 2006), was found nested within a group composed of European and Canadian specimens of *D. scoparium* (BP = 100, PP = 1). As *D. johnstonii* and *D. scoparium* are morphologically very similar, sharing 2-4 abaxial lamella, porose cells, upper margin with teeth, we suggest to consider *D. johnstonii* as a synonym of *D. scoparium*. However, the worldwide distributed *D. scoparium* is unknown from sub-Saharan region (O'Shea, 2006) (Fig.1) and the occurrence of the species on Kilimanjaro Mountains (relict populations or long dispersal) is not explained, yet. This result shows that the distributional range and the dispersal ability of *D. scoparium* have been underestimated, despite more and more evidence of bryophytes long dispersal capabilities have been recently found (Grundmann & *al*., 2007, Devos & Vanderpoorten, 2010). Interestingly, the ecology and distribution of the remaining African species is poorly known, and sampling efforts combined to morphological revisions will presumably show similar patterns. Interestingly, Canadian and US populations of *D. scoparium* did not belong to the same group, and further investigation combining morphology and molecular studies is needed to understand this morphologically variable taxa.

The present study both validates the "traditional view" of bryophyte as "unmoving, unchanging sphinxes of the past" (Crum, 1972) with species sharing low genetic variability between populations separated on different continents (see *D. polysetum, D. undulatum*, Fig.4), and that the bryophytes share the same genetic diversity as vascular plants, and their morphological uniformity masks underlying genetic complexity (see *D. majus* subspecies, Fig. 4; Shaw, 2001).

4.3.2 Systematic concepts

Species belonging to genera *Dicranum*, *Paraleucobryum* and *Pseudochorisodontium* clearly form a monophyletic group (BP = 100, PP = 1, Fig. 4) confirming previous molecular analysis based on trnL-trnF and rps4 sequences (Lafarge & *al.*, 2002). However, their relationship remains unresolved. The addition of genus *Chorisodontium* and the use of more variable markers would help to resolve the backbone of *Dicranum* s. l.

The polyphyly of genus *Pseudochorisodontium* call into question the validity of criteria for identifying this group of Chinese-Indian endemic species (i.g. lack of teeth on the peristome *in* Gao & Cao, 1999). The presence of this unique character in Dicranoideae appears to be homoplasic, and can be due to convergent morphological evolution. Reduction or absence of peristome is considered as an adaptation to the epiphytic habitat which always has at least some xeric tendencies (Vitt, 1981). This assumption is challenged here. In fact, five of the six *Pseudochorisodontium* species grow on soil or soil over rock (Gao & Cao,

1999), sometimes in wet areas as in the Mount Gongga forests (Sichuan Province, China). Thus, according to the weather station at 3000 m (where studied samples were collected) the mean annual precipitation is 1925 mm, most (approx. 80%) falling between June and October, with, mean monthly temperature ranges from -4.5°C in January to 12.7°C in July (Huo & *al*., 2010). The type species, *P. gymnostomum*, being nested into *D. scoparium sensu lato* (ML = 92, BP = 1, Fig. 4), we therefore suggest to consider *Pseudochorisodontium* as a synonym of *Dicranum*. However, before any nomenclatural changes, further investigation is needed on the six syntypes describing the type species as they do not seem consistents (data not shown).

Genus *Orthodicranum* as described by Loeske (1910) is paraphyly, *D. setifolium* being nested in a reliable clade (BP = 85, PP = 1) including *D. flagellare, D. tauricum, D. viride, D. fulvum*, and *D. montanum*, except Asian *D. tauricum*. This result confirms the taxonomic treatment of Bellolio-Trucco & Ireland (1990) who have shown that the morphological characters describing *Orthodicranum* (erect, smooth or slightly furrowed capsules, unistratose alar cells, and lower cells non pitted or weakly pitted) were variable throughout the genus and tend to grade into the boundaries set for the genus *Dicranum*. Again, before any nomenclatural changes, the type species that has not been designated by Bruch and Schimper (1851) has to be found and studied. More challenging, from the outset, the validity of the genre *sensus* Loeske seems to be problematic since the author himself said the genus is may be just temporary ("ist vielleicht nur provisorich", p.85, Loeske, 1910). He wonder if the erect capsule alone is a good character to discriminate the species from *Dicranum*, and add that he has to study closely-related *Leiodicranum* and *Crassidicranum* sections before any changes. Nomenclatural investigations are clearly needed both to designate a type species and to validate or not the taxonomic level of "Orthodicranum".

The numerous polyphyletic species found in *Dicranum* must be put into perspective by mentionning that during the period of active bryological exploration of extra-European regions during the ninteenth century, hundreds of new "geographical species" were described based in large part on the assumption that populations from distant regions must represents different species distinct from familiar European taxa (Shaw, 2001). One can assume that a taxomonic revision of the extra-European endemic type specimens would reduce the number of species by synonymizing them. More problematic is the incongruence between type specimen morphology and literature, especially in under-studied areas such as Asia (except Japan) or Africa. For example, *Dicranum japonicum* differs from *D. lorifolium* by male dwarfism according to the Chinese Moss Flora, but here we observed several dwarf males on the type specimen PC0128679.

Conclusions

This study gives insights into the molecular relationships that challenge the long established concepts of the morphologically defined species in *Dicranum,* revealing polyphyletic and cryptic species. Mosses exhibit fairly simple morphologies, whose transformation may be rather cryptic, and hence fewer characters to infer relationships (Goffinet & Vanderpoorten, 2006). Species delimitation was found to be problematic for *D. scoparium* and, more in general, for most Asia and Africa taxa. Nomenclatural uncertainties, literature incongruence, old concepts of species delineation explain, among other, these results. Several reproductive traits have been mapped on the phylogenetic species tree revealing homoplasy, and troubling species identification. Insights into the evolution of male dwarfism are promising but challenging has *D. scoparium* and *D. bonjeanii* have both size males. This then, requires the observation of each sample studied to determine the size of the male plants. Lack of a worldwide comprehensive morphological study of *Dicranum* further complicates the situation.

Additional molecular data have to be gathered, in connection with careful morphological analysis and type specimens revision. In addition, sampling must be broadened, in particular of East Asian, Boreal, Oceanian and African taxa, in order to arrive at a better understanding of phylogenetic relationships and classification of the genus. The phylogeny presented here will hopefully be a fruitful basis for future studies on the evolution of the genus.

Acknowledgements

We would like to thanks all the people who provided plant material; these include Bernard Goffinet, Claudine Ah-Peng, Min Thuan, Jean Faubert, Lars Hedenäs, Michael Ignatov, and his daughter, Nele Ingerpuu, Masanobu Higushi, Sanna Laaka-Lindberg, Angela Newton, Cecilia Sergio, Scott Schuette, Bruno de Reviers, Frederic Ritz, Alain Untereiner, Serge Müller, Torbjörn Tyler, Simon Laegaard, Jacques Bardat. Thanks are also due to the directors/curators of the following herbaria for the loan of herbarium specimens and permission to take tissue samples: S (Lars Hedenäs), MW (Michael Ignatov), TNS (Masanobu Higushi), G (Michelle Price), and PMA (Noris Salazar Allen). We are grateful to Alain Untereiner, Vincent Hugonnot, Frederic Ritz, Jean-Claude Vadam, Cao Tong, Martin Nebel for their help and welcome during the different fieldtrips, Jacques Florence for help in resolving nomenclatural problems and to Amandine Allard for assistance in checking and digitalizing the *Dicranum* types in herbarium PC. We also thank Robbert Gradstein for helpful comments on the manuscript.

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Tables

Table 1: Amplification and alignment length, number of variable sites, number and percentage of parsimony informative characters included in the analysis

Table 2: Number of specimens shared in each of the 143 haplotypes.

Table 3: Primers used to amplify the specimens of this study

Figures

Figure 1: World distribution of the genus *Dicranum* s.str. compiled from: America: He, 1998 ; Worley & Iwatsuki, 1970 ; Thériot, 1932 ; Menzel, 1992 ; Bellolio-Trucco & Ireland, 1990; Florschütz-De Waard & Florschütz, 1979; Hermann, 1976; Golberg, 2003 ; Africa: Bizot, 1973 ; Demaret, 1940 ; Jelenc, 1949-1953 ; Oceania: Brown, 1896 ; Norris & Kopponen, 1990 ; Asia: Iwatsuki, 2004 ; Osada, 1958 ; Higishi & Nishimura, 2003 ; Noguchi, 1954 ; Redfearn & Wu, 1986 ; Gao & Cao, 1992 ; Mitten, 1859 ; Thériot ; 1918 ; Frey & Kürschner, 2009 ; Müller, 2009 ; El-Oqlah & al., 1988 ; He, 1996 ; Ninh, 1993 ; Lin, 1981 ; O'Shea, 2003 ; Europe: Colacino & Sabovljevic, 2006 ; Natcheva & Ganeva, 2005 ; Ignatov & Afonina, 1992 ; Hill & al, 2006 ; Casas & al., 2006 **;** Bergþór, 2003.

Figure 2: 50 % majority-rule consensus tree of *Dicranum* based on sequences of four genes (*rbc*L, *rpl*32-*trn*L, *Gap*C, *Nad*5-4), obtained from the bootstrap analysis (1000 replicates) under the maximum likelihood method. Numbers above branches indicate bootstrap values in (1) supermatrice bootstrap (BP), (2) superTri bootstrap (SBP), (3) mean bootstrap (MBP) under superTRI. The four squares at the nodes summarize the results of the independent analysis of the four markers, from left to right: *rbc*L, *rpl*32-*trn*L, *Gap*C and *Nad*5-4. A green square indicates that nodes were supported by BP > 50; a white square indicates low support (BP < 50); a red square indicates that a different relationship is supported by BP > 50. BP value is indicated in each square. (-) node not found in the analysis, (*) indicate more than one sample sharing the haplotype

Figure 3: 50 % majority-rule consensus tree of *Dicranum* based on sequences of four genes (*rbc*L, *rpl*32-*trn*L, *Gap*C, *Nad*5-4), obtained from the posterior probabilities analysis (PP) under the Bayesian inference method. Numbers above branches indicate (1) supermatrix posterior probabilities (PP), (2) superTri posterior probabilities (SPP), (3) mean posterior probabilities (MPP) under superTRI. The four squares at the nodes summarize the results of

the independent analysis of the four markers, from left to right: *rbc*L, *rpl*32-*trn*L, *Gap*C and *Nad*5-4. A green square indicates that nodes were supported by PP > 0.75, a white square indicates low support (PP < 0.75); a red square indicates that a different relationship is supported by PP > 0.75. PP value is indicated in each square. (-) node not found in the analysis, (*) indicate more than one sample sharing the haplotype

Figure 4: Phylogram resulting from Bayesian analysis. Green bold lines represent very reliable nodes, i.e., which were supported by supermatrix and SuperTri methods, at least two independent genomes, and BI and ML analysis; blue regular lines represent reliable nodes, i.e., which were supported by supermatrix and SuperTri methods, and BI and ML analysis; brown dotted lines represent unreliable nodes, i.e., which were supported by supermatrix and SuperTri methods, supported by one markers but contradicted by on another marker in separated analysis; red lines represents strong contradicting nodes, with robust contradiction between supermatrix and SuperTri methods, and robust contradiction between combined and separated analysis.

Figure 5: Mapping of morphological and sexual traits on cladogram resulting for the ML and BI analysis. Bold lines represent very reliable nodes, which are supported by supermatrice and superTri methods, two independent genomes, and BI and ML analysis; regular lines represent reliable nodes which are supported by supermatrice and superTri methods, and BI and ML analysis. Violet lines represents the occurrence of dwarf males, orange lines represents the occurrence of normal-sized males; green leaves represent the production of flagelliform branches; blue sticks represent the occurrence of caducous leaves; brown circle represents the production of gemmae; cross represents taxa without peristome teeth.


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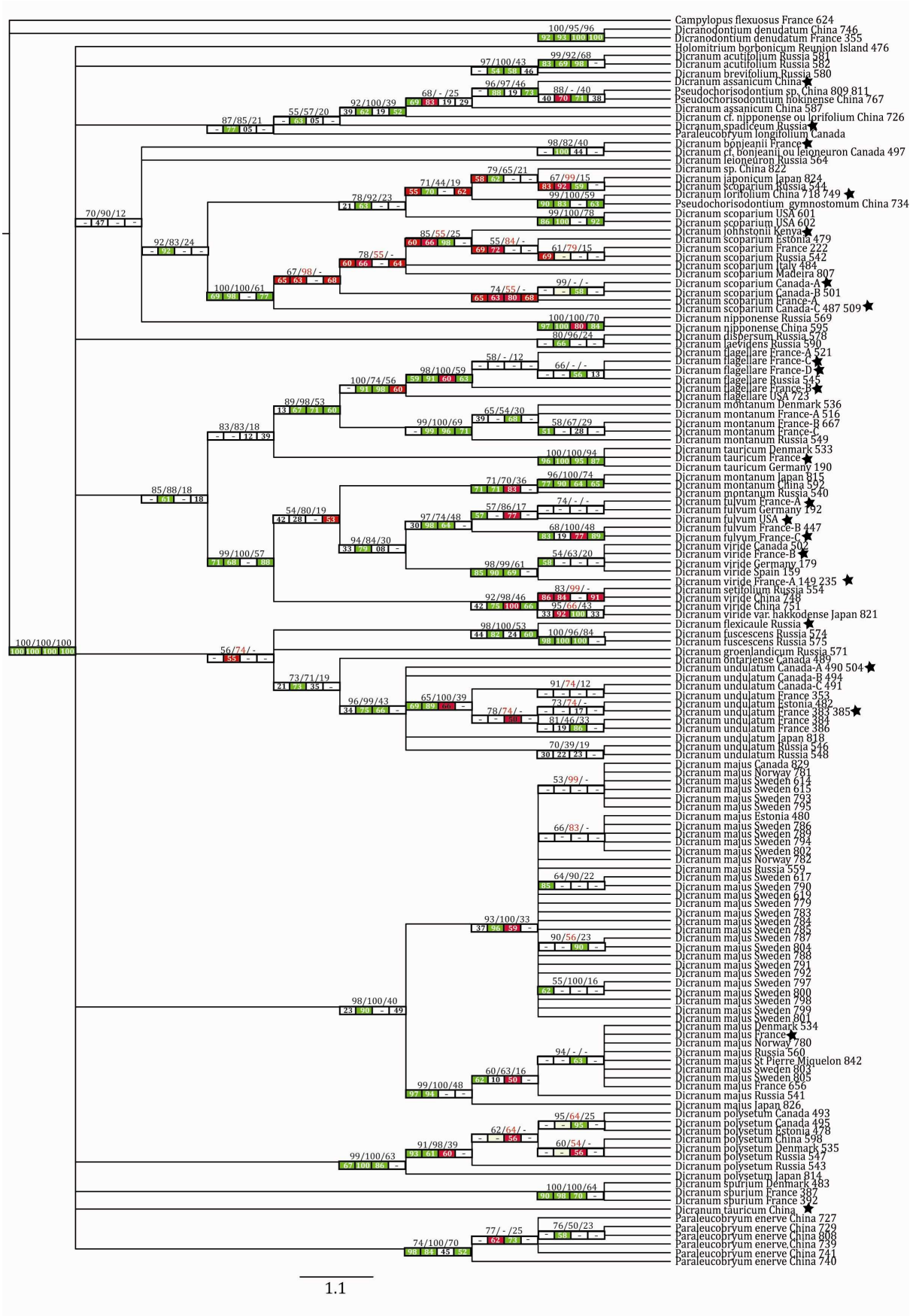
Produced by the Cartographic Research Lab
University of Alabama

Figure 1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Dicranum cf. bonjeanii ou leioneuron Canada 497
Dicranum leioneuron Russia 564
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Figure 2

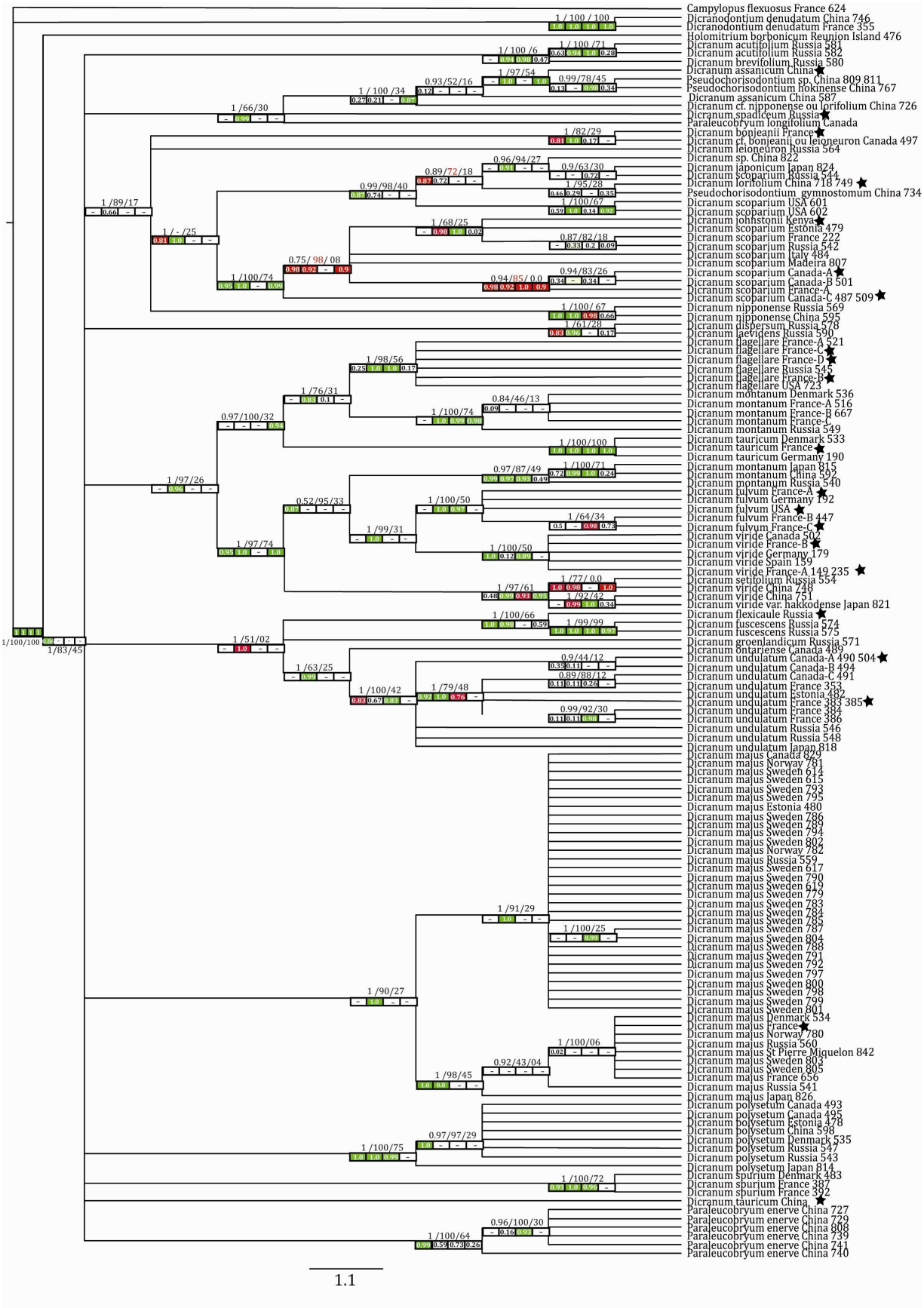
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Figure 3

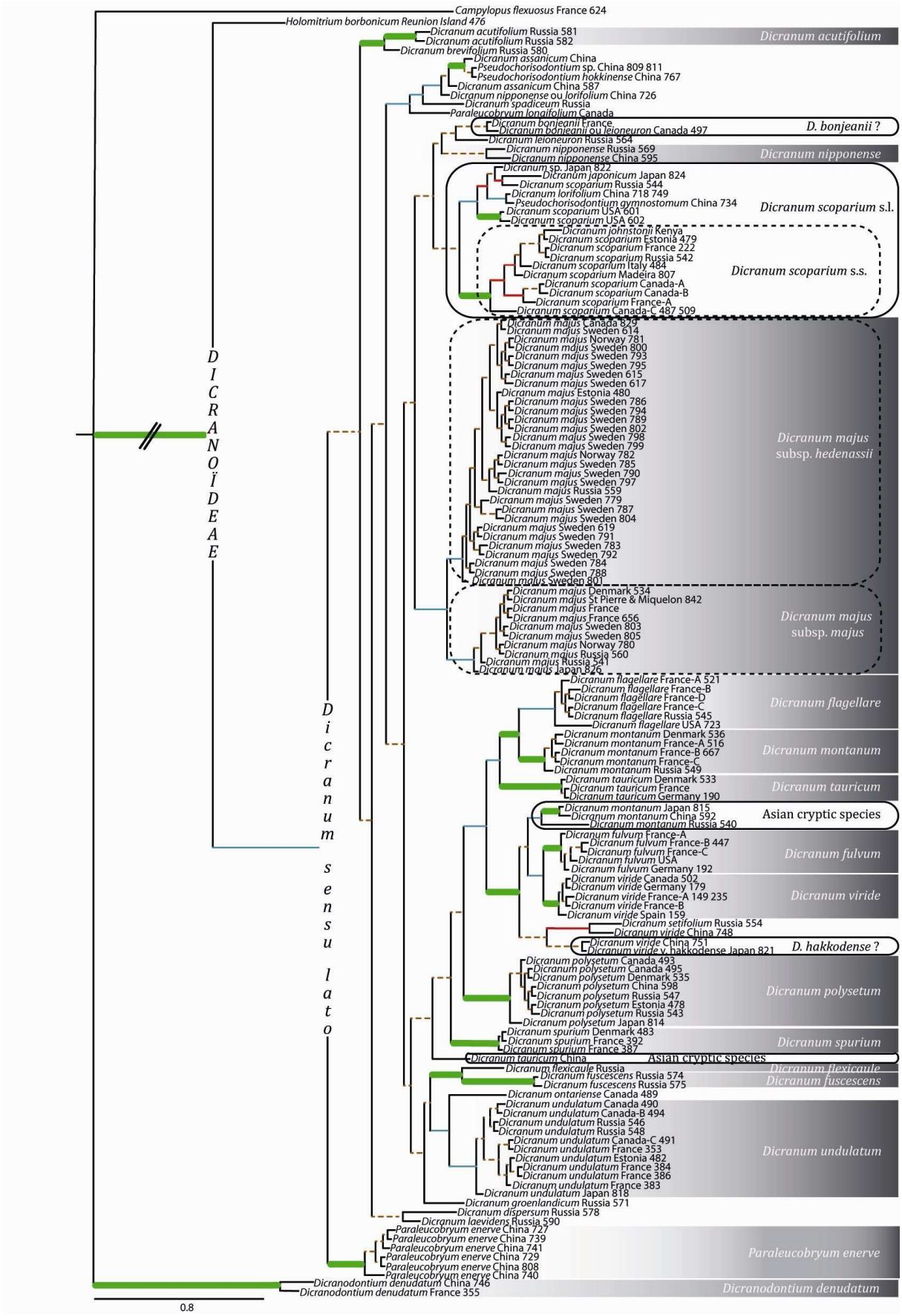
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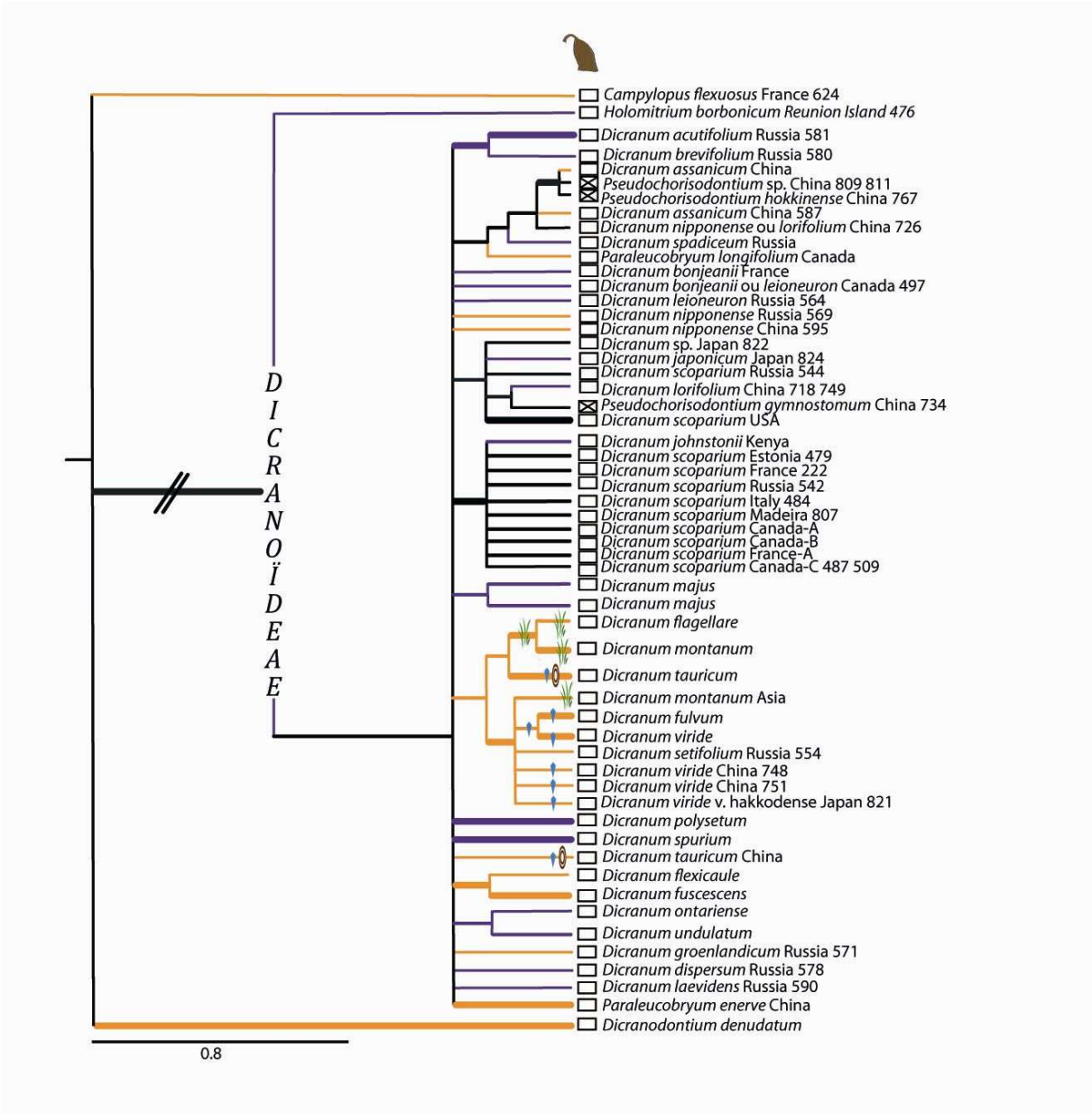
Figure 4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Dicranum scoparium Canada-B
Dicranum scoparium France-A
Dicranum scoparium Canada-C 487 509
Dicranum majus
Dicranum majus
Dicranum flagellare
Dicranum montanum
Dicranum tauricum
Dicranum montanum Asia
Dicranum fulvum
Dicranum viride
Dicranum setifolium Russia 554
Dicranum viride China 748
Dicranum viride China 751
Dicranum viride v. hakkodense Japan 821
Dicranum polysetum
Dicranum spurium
Dicranum tauricum China
Dicranum flexicaule
Dicranum fuscescens
Dicranum ontariense
Dicranum undulatum
Dicranum groenlandicum Russia 571
Dicranum dispersum Russia 578
Dicranum laevidens Russia 590
Paraleucobryum enerve China
Dicranodontium denudatum
DICRANOÏDEAE
0.8

Figure 5

Table 1

	Length of PCR products	Alignment length	Number of variable sites	Number of parsimony informative characters	Percentage of parsimony informative characters
*rcb*L (cp)	771-790	791	93	71	9
*rpl*32-*trn*L (cp)	496-587	658	185	125	19
*Nad*5-4 (mt)	753-763	767	61	51	7
*Gap*C (nr)	286-505	589	167	115	20
ITS1 (nr)	287-515	606	Ø	Ø	Ø

Table 2

Haplotype per country	**Number of specimens sharing the haplotype**
acutifolium (russia)	1
acutifolium (russia)	1
assanicum (china)	3
bonjeanii (France)	2
bonjeanii (Russia) = leioneuron	1
brevifolium (Russia)	1
dispersum (russia)	1
drummondii (China) = assanicum	1
flagellare (France A)	1
flagellare (France B)	4
flagellare (France C)	3
flagellare (France D)	8
flagellare (Japan) = montanum	1
flagellare (Russia) = montanum	1
flagellare (russia)	1
flagellare (USA)	1
flexicaule (russia)	2
fulvum (France) A	3
fulvum (France) B	1
fulvum (France) C	3
fulvum (Germany)	1
fulvum (USA)	2
fuscescens (Russia)	1
fuscescens (Russia)	1
groenlandicum (russia)	1
japonicum (Japan) = Dicranum sp.	1
johnstonii (kenya)	1
laevidens (russia) A	1
majus (Canada)	1
majus (Denmark)	1
majus (Estonia)	1
majus (France)	31
majus (Japan)	1
majus (norway)	1

majus (norway)	1
majus (norway)	1
majus (Russia)	1
majus (Russia)	1
majus (Russia)	1
majus (St pierre & Miquelon)	1
majus (Sweden)	30 haplotypes
montanum (China)	1
montanum (Denmark)	1
montanum (FranceA)	1
montanum (FranceB)	1
montanum (FranceC)	1
montanum (Russia)	1
nipponense (China) = nipponense or lorifolium	1
nipponense (Japan) = japonicum	1
nipponense (Russia)	1
ontariense (Canada)	1
Para. longifolium (Canada)	2
Para. enerve (china)	1
Para. enerve (china)	1
Para. enerve (china)	1
Para. enerve (china)	1
Para. enerve (china)	1
Para. enerve (china)	1
polysetum (Canada)	1
polysetum (Canada)	1
polysetum (Canada) = bonjeanii or leioneuron	1
polysetum (China)	1
polysetum (Denmark)	1
polysetum (Estonia)	1
polysetum (japan)	1
polysetum (russia)	1
polysetum (russia)	1
Pseudo conananeum (China) = P. hokinense	1
Pseudo gymnostomum (China)	1
scoparium (CanadaA)	6
scoparium (CanadaB)	1
scoparium (CanadaC)	2
scoparium (china) = lorifolium	2
scoparium (China) = Pseudo. sp.	2
scoparium (China) = nipponense	1
scoparium (Estonia)	1
scoparium (France)	1
scoparium (France) A	1
scoparium (Italy)	1
scoparium (Madeira)	1
scoparium (Russia)	1
scoparium (Russia)	1
scoparium (USA)	1
scoparium (USA)	1
setifolium (Russia)	1
spadiceum (russia)	2

spurium (Denmark)	1
spurium (France)	1
spurium (France)	1
tauricum (china)	16
tauricum (Denmark)	1
tauricum (France)	1
tauricum (Germany)	1
undulatum (CanadaA)	2
undulatum (CanadaB)	1
undulatum (CanadaC)	1
undulatum (Estonia)	1
undulatum (France)	1
undulatum (France)	1
undulatum (France)	1
undulatum (France)	2
undulatum (Japan)	1
undulatum (russia)	1
undulatum (russia)	1
viride (Canada)	1
viride (china)	1
viride (china)	1
viride (France) A	2
viride (France) B	5
viride (germany)	1
viride (Spain)	1
viride var hakko (japan)	1

Table 3

Genome	Gene	Primer name	Sequence (5'-3')	Reference
cp	*rbcL*part 1	*rbc*L-TKT-F1 (F1_F)	ACCCAWGTCACCACAAACRGAG	Ebihara *etal.* BotJLinSociety (2007)
		TKT-F1-TKT-2PRN (2PRN-R)	CGTTCTCCTTCCAGTTTRCCTACTACAGT	Ebihara *et al.* (2007)
		rbcL_300S	AAAGCTTTACGAGCCTTGCGTCTAGAA	
		rbcL_400AS	ATCCCAATAATGGACGACCATATTTG	
		rbcl_600S	TCCCAAGCTGAAACAGGTGAAATTA	
		rbcl_800AS	TCAATAACTGCATGCATTGCACGGT	
	*rpl*32-*trn*L	rpl32	ACCCAWGTCACCACAAACRGAG	Shaw *et al.*(2007)
		trnL	CGTTCTCCTTCCAGTTTRCCTACTACAGT	Shaw *et al.* (2007)
		R32_200S	TATATAGCACCTAACAATTAAATGT	
		R32_500AS	TCAATTTTAATGGGGCATAGCGTAG	
mt	*Nad* 5-4	Nad5-4S	GGAATTTCGTACACATTTCG	
		Nad5-4AS	AATACTTCGTATCAGTCGTA	
		Nad5-4_300S	AGCCTAAGCTATGCTTTGCGGTCTA	
		Nad5-4_400AS	ATCATTCCATCATTCCAACCTTTA	
		Nad5-4_600S	TAGTATGCGCGTATAAAAGCTCATC	
		Nad5-4_750AS	ACTCTTTTTCCAAAAGATCCCGAGGA	
nr	*Gap*C	GapC_E7S	TGCCAAGGTTATCAACGACAAGTTCG	
		GapC_E10AS	CGGTGTAACCCAARATRCCCTTCATC	
	ITS 1	Its5-bryo	GGAAGGAGAAGTCGTAACAAGG	
		itsC-bryo	GCAATTCACACTACGTATCGC	

Chapitre II : Variabilité et dispersion : le cas de *Dicranum majus* Turn.

Article 3 : Pichonet, A., Hassanin, A., Cruaud, C., Couloux, A., Gradstein, R., Bardat, J. *Dicranum majus* Turn., two species into one? The signal of five genomic regions. To be submitted to Plant Systematics and Evolution

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Dicranum majus Turn., two species into one? The signal of five genomic regions

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Abstract *Dicranum majus* is a morphologically variable taxon. Recently, two phenotypes of *D. majus* from Europe have been described: a southern phenotype occurring from France to southern Sweden, and a northern phenotype occurring from southern Sweden to northern Scandinavia. In the present study we have tested the genetic basis of the two phenotypes using five markers from three genomes (plastic *rbc*L and *rpl*32-*trn*L, mitochondrial *Nad*5-4 and nuclear ITS1, *Gap*C exon 6-exon10). *Dicranum majus* was found to be monophyletic with low support in the combined analysis but with strong support in the separate analyses. The two phenotypes were recovered with strong support. One accession from Sweden formed a third molecular group and two specimens from Canada were morphologically intermediate. Based on the results, the two phenotypes are described as geographical subspecies, subsp. *majus* and subsp. *hedenaesii* subsp. nov. The worldwide morphological circumscription and the status of morphological varieties described in *D. majus* need further study.

Keywords: *Dicranum majus*; Dicranaceae; phylogeny; subspecies; phenotypic plasticity; Europe

1. Introduction

Dicranum majus Turn. (Dicranaceae) is a circumpolar acrocarpous bryophyte (Fig. 1) occurring on humus, soil and rotten wood in coniferous and deciduous forests, bogs and tundra throughout the Northern Hemisphere, from sea level to 4500 m (Briggs, 1965; Bellolio-Trucco & Ireland, 1990; Dierssen, 2001). In its typical form the species is recognized by its tall stems (10-15 cm) and its glossy, long (6-12 mm), falcate-secund leaves that are irregularly serrate to weakly ridged on the dorsal surface of the costa (Crum & Anderson, 1981) (Fig.2). The presence of a double row of guide cells, aggregate setae, rectangular lamina cells, long leaves which are strongly and uniformly curved to one side, and the narrow costa (1/9 of the leaf base), separate *D. majus* from allied species of the section *Dicranum* such as *D. scoparium* Hedw., *D. polysetum* Sw., *D. crassifolium* Sérgio, Ochyra & Seneca, *D. transilvanicum* Luth, *D. bonjeanii* De Not. and *D. leioneuron* Kindb. (Briggs, 1965; Bellolio-Trucco & Ireland, 1990; Nyholm, 1954).

Dicranum majus is a very variable taxon; for example, in the mountains of Scandinavia small forms occur with erect, nearly smooth leaves, upper leaf cells nearly quadrate (Nyholm, 1954), and a costa with only one row of guide cells (Briggs, 1965). Eleven varieties have been described in the species, six of which are still being accepted: *D. majus* var. *capnodes* I. Hagen, *D. majus* var. *condensatum* I. Hagen, *D. majus* var. *minus* Brusch. & Schimp., *D. majus* var. *orthophyllum* A. Braun ex. Milde, *D. majus* var. *subundulatum* Warnstand and *D. majus* var. *undulascens* Kindb. The remaining ones have been synonymised with other species of *Dicranum.*

Recently, two phenotypes of *D. majus* from Europe were described by Hedenäs & *al.* (2006): a southern phenotype occurring from France to southern Sweden, and a northern phenotype occurring from southern Sweden to northern Scandinavia. The ranges of the two phenotypes overlap in southern Sweden. The two phenotypes differ by (1) leaves short and straight to slightly curved (northern phenotype) or long and falcate (southern phenotype), (2) upper leaf lamina unistratose and smooth (northern phenotype) or with a bistratose, submarginal band and dorsally rough by spine-like projections (southern phenotype), and (3) costa dorsally rough only near apex and with one layer of guide cells (northern phenotype) or rough down to mid-leaf and with two layers of guide cells (southern phenotype) (Fig.2). The authors concluded that the southern plant with strongly falcate leaves is clearly the one originally described and illustrated as *D. majus* by Turner (1804) and other authors (e.g., Smith, 1804). Hedenäs & *al.* (2006) also found that type material of *D. majus* var. *orthophyllum* A. Braun ex Milde belongs to the southern phenotype. The question whether the

two forms were genetically differentiated taxa or were just environmental modifications remained unknown, however, and is the subject of this paper.

Patterns in genetic diversity within bryophyte species has been studied by a variety of molecular tools, varying from isozymes (e.g., Szweykowski & *al.*, 1979) to DNA sequences and microsatellites (e.g., Vanderpoorten & *al*., 2001; van der Velde & Bijlsma, 2000), for a review see Shaw (2008). Using molecular techniques, numerous paraphyletic species (e.g., Stech & Dohrmann, 2004) or morphologically cryptic groups (e.g., Shaw & Scheiner, 1995) have been detected within bryophytes. The complex interspecific evolutionary patterns recovered, including cryptic speciation, hybridization, and allopolyploidy, contradict Crum's (1972) claim that bryophytes represent "unchanging, unmoving sphinxes of the past" (Shaw, 2008). However, it should be noted that only few studies employed experimental approaches to determine the base of the morphological variation (but see, for example, Hedenäs, 1996; Buryova & Shaw, 2005; derpoorten & Jacquemart, 2004).

A first molecular-phylogenetic study of *Dicranum*, based on a limited sampling of the genus (but including *D. majus*) and using two chloroplastic markers (trnL-trnF, rps4), was carried by Lafarge & *al.* (2002). This study failed to resolve phylogenetic relationships at the species level within the genus *Dicranum*. The question whether *D. majus* is monophyletic or not remained unclear.

In the present study we used five markers from all three genomes to investigate the genetic structure of *Dicranum majus*, with a special focus on European populations. The aims of our study were to test (1) the genetic basis of the two geographical groups in Europe (Hedenäs *& al.,* 2006), and (2) the monophyly of the species.

2. Material and methods

2.1. Specimen identification and taxon sampling

Twenty-eight specimens of the northern phenotype and 41 specimens from the southern phenotype of *D. majus* (Hedenäs & *al.*, 2006) were used for this study. About half of the specimens were freshly collected in the field, the remaining ones were herbarium specimens from S, MW, and TNS. "Blind" identification of the two phenotypes was conducted by J.B and Dr. L. Hedenäs on 7 specimens (Table 1), in order to test the reliability of the morphological characters and the distinctiveness of the two phenotypes. The geographical origin of the samples is shown in Fig. 1; voucher information is given in the Appendix. Specimens from *Dicranum scoparium, D. muehlenbeckii* Bruch & Schimp., *D. polysetum* and *Holomitrium borbonicum* Hampe ex Besch. were selected as outgroups on the

basis of the molecular phylogeny of the Dicranoideae by Lafarge & *al.* (2002).

Stems from which DNA was extracted were placed in an Eppendorf with silicagel and returned to the convolute containing the whole specimen. Each stems was carefully scrutinized for possible occurrence of epiphytic dwarf male plants; caution was taken to avoid contamination by DNA of dwarf males. When DNA contamination by dwarf males was suspected (e.g. in the case of incongruent results), another shoot from the same sample was analysed according to the same protocol. When identical, only one sequence per sample was included in the final matrix data set.

2.2 Genomic sampling and alignment

2.2.1 Marker choice and primer design

As recommended by the CBOL Plant Working group (2009), a combination of genomic regions including barcode standards was analysed. Two chloroplast regions (*rbc*L, *rpl*32-*trn*L$^{(UGA)}$), one mitochondrial region (*Nad*5-4) and two nuclear regions (ITS1, *Gap*C exon 6-exon10) were selected for molecular characters.

Primers for amplifying and sequencing the ITS region (ITS5-bryo and ITSC-bryo) were based on Sabovljevic & *al.* (2005). Primers for amplifying and sequencing the *rbc*L region (F1_F and 2PRN_R) were based on Ebihara & *al*. (2007), and primers for amplifying and sequencing the *rpl*32-*trn*L$^{(UGA)}$ region (*rpl*32 and *trn*L) were based on Shaw & *al*. (2007). Internal primers were designed for *rbc*L and *rpl*32-*trn*L$^{(UGA)}$ with respect to bryophytes. Primers for amplifying and sequencing the *Gap*C and *Nad* 5-4 regions were designed for this study using available sequences of the genus *Dicranum* and close related genus on NCBI. Primers amplifying the *Nad*5-4 region were designed with sequences from *Orthodicranum, Leucobryum, Ulota, Splachnum, Orthodontium, Aulacomnium, Pohlia, Mnium, Plagiopus, Bartramia, Rhacocarpus, Fontinalis,* and *Pterogonium* genus. Downloaded representatives for *Gap*C primers design included *Marchantia, Sphagnum, Physcomitrella* and *Pachycladon* genus. Although Szövényi & *al.* (2006) found *Gap*C to be amplified in single-copy in *Sphagnum,* paralogue genes have been sequenced for *Marchantia* and *Physcomitrella* (DQ873396, AJ246025, AJ246024*).* When possible, both paralogues (i.g *Gap*C and *Gap*Cp) were included in the alignment (*Marchantia polymorpha* and *Physcomitrella patens),* and the exon-intron structure between exon 6 and exon 10 was reconstructed based on the annotated sequences of *Sphagnum* ssp. and the downloaded range of bryophyte and vascular plant sequences available on NCBI.

During the PCR reaction a high hybridization temperature (65 °C) was used to avoid any contaminants (e.g. no selective hybridization). Ambiguous bases or double peaks, which may indicate that more than one copy of the gene was amplified, were checked on sequencing chromatograms. The primers are shown in Table 3.

2.2.2 DNA extraction and PCR conditions

Plant DNA from distal stem was extracted from fresh material using Quiagen Plant DNA Kit. Each sample was ground with two steel balls in a 2 mL tube for 2 min 30 s at 25 Hz in (Retsch Mixer Mill 300) .The buffer AP1 is modified by mixing (for 24 samples): 12 mL AP1; 0. 005 g BSA; 1,64 g of sucrose; 0. 48 g of SDS, and 24 µL of betamercaptoethanol. The step 1 was modified as following for the herbarium specimens: stems were lysed with 400 µL of AP1, 30 µL of CTAB and 30 µL of proteinase K for 24 hours at 42°C.

The standard PCR amplification conditions were as follows: (*rbc*L) initial denaturation at 94°C for 1 min; 40 cycles of denaturation at 94°C for 30 s, anneling at 49°C for 40 s, extension at 72°C for 40 s; final extension at 72°C for 5 min. (*rpl*32-*trn*L, see Shaw *& al.*, 2007) (*Gap*C) initial denaturation at 94°C for 1 min; 10 cycles of denaturation at 94°C for 30 s, anneling at 65°C for 1 min, extension at 72°C for 1 min, 35 cycles of denaturation at 94°C for 30 s, anneling at 60°C for 1 min, extension at 72°C for 1 min; final extension at 72°C for 5 min.

(ITS 1) initial denaturation at 94°C for 1 min; 10 cycles of denaturation at 94°C for 30 s, anneling at 55°C for 1 min, extension at 72°C for 1 min, 30 cycles of denaturation at 94 °C for 30 s, anneling at 50°C for 1 min, extension at 72°C for 1 min; final extension at 72°C for 5 min. (Nad5-4) initial denaturation at 94°C for 1 min; 40 cycles of denaturation at 94°C for 30 s, anneling at 60°C for 1 min, extension at 72°C for 1 min; final extension at 72°C for 5 min.

PCR were purified using Quiagen kit and both strands were sequenced on ABI 3130. Thermocycler settings for the sequencing PCR were as follows: initial denaturation at 96°C for 1 min; 25 cycles of denaturation at 96°C for 10 s, anneling at 50°C for 5 s, extension at 60°C for 4 min. Some specimens were also sequenced by Genoscope.

Contigs were constructed from single-stranded forward and reverse sequences using Sequencher 3.0 (Gene Codes Corp.) and CodonCode Aligner (Codon Code Corp.). Sequences were aligned manually on SeAl v2.0a11 (Rambaut, 2002) and double check by eyes. Gaps were inserted where necessary to preserve positional homology.

2.3 Phylogenetic analysis

2.3.1 Model choice

The use of appropriate models is essential if we are to be confident in the results of a phylogentic analysis as they affect all aspects of phylogenetic analysis (Posada & Buckley, 2004). Several strategies for model choice have been proposed in the context of phylogenetics (i.e. hLRT, BIC, AIC) (see Huelsenbeck & *al.*, 2004; Posada & Buckley, 2004 for reviews). Here, we selected the best models fitting the data set (separated and combined) based on the Akaike information criterion (AIC, Akaike, 1973, 1974) which has the advantages of being able to simultaneously compare multiple nested or nonnested models, account for model selection uncertainty, and allow for model-average inference (Posada & Buckley, 2004). Model selection was done using the program Modeltest (Posada & Crandall, 1998).

2.3.2 Bayesian analysis

Phylogenetic analysis of DNA sequences were conducted by Bayesian inference under the assumptions of a model of nucleotide substitution. The data set partitions were analyzed separately to test the existence of conflicting signals. The absence of incongruence between *rbc*L, *rpl*32-*trn*L and *Nad*5-4 enable us to concatenate them manually, but GapC and ITS1 were analysed separately. Informative indels were coded. The models fitting the best the combined matrix (GTR + I + G), GapC (HKY + G) and ITS1 (HKY) were conducted on MrBayes 3.1.2. Four chains of 2,000,000 iterations were run and trees were sampled every 100 generations to ensure independence of successive trees. The 10,000 trees of the 'burn-in' for each run were excluded from the tree set.

2.4 Morphological character scoring

From each of the two major molecular groups, twelve specimens were randomly selected, and ten lower leaves cross sections, ten upper leaves cross sections, and five entire leaves were examined, from each specimen. Six morphological characters used for phenotype delineation in *D. majus* (Hedenäs *& al.*, 2006) and five additional morphological characters were measured (Table 2). Morphological characters used by Hedenäs *& al.* (2006) were slightly modified, including the number of guide cells, the number of projecting cells and the bistratification of the leaf. Leaf length in each molecular group was analysed in box plots, and a Wilcoxon-Mann-Whitney test was conducted to examine morphological similarities.

3. Results

3.1 PCR amplification and data set construction

Length variation, alignment length, amplification success, number of variable and parsimony informative characters are given in Table 4.

In total, 95 *Gap*C, 91 ITS1, 94 *rbc*L, 91 *rpl*32-*trn*L and 91 *Nad*5-4 sequences were amplified and sequenced (Table 4). The success of PCR amplification was 96% for ITS 1, 99% for *rbc*L, 96%, for *rpl*32-*trn*L and *Nad*5-4, and 100% for *Gap*C. For the *Gap*C region, sequencing chromatograms showed no ambiguous bases or double peaks, which may indicate that only one copy of the gene was amplified.

Only the specimens for which all five markers were successfully sequenced were included into the final alignment, except n° 576 (*Nad*5-4 missing) and n° 796 (*rpl*32-*trn*L missing).

3.2 GapC Structure

The intro-exon structure of *Gap*C/*Gap*Cp is illustrated in Figure 3. The investigation of the *Nad*5-4 spacer structure show loss of introns in different lineages compared to *Sphagnum* genus. Within Bryophyta, the genera *Syrrhopodon*, *Mitthyridium* and *Dicranum* differed from *Sphagnaceae* by lacking intron (i) 6, 8 and 9. Furthermore, *Gap*Cp differed from *Gap*C by the loss of i8 and i9, in the liverwort *Marchantia polymorpha*; whereas, in the moss *Physcomitrella patens,* i6 is conserved and i8 lost in *Gap*C compared to *Gap*Cp.

3.3 Phylogenetic information of the markers

Total length, variable and parsimony-informative sites were summed up in Table 4. The alignment lengths of the cytoplasmic combined matrix (*Nad*5-4 – *rpl*32-*trn*L – *rbc*L), ITS1, and *Gap*C were respectively 2,975, 388, and 498 nucleotide characters. Percentages of parsimonious sites vary from 0 % (*Nad*5-4) to 2.76% (*rpl*32-*trn*L). Within the ingroup ITS1, *rbc*L and *Nad* 5-4 were free from indels.

3.4 Phylogenetic results

In the Bayesian tree (Fig.4), *Dicranum majus* was found to be monophyletic but with low support (PP = 0.71) and subdivided into two molecular groups, respectively called group A, and B. Group A included, in majority, specimens from Sweden and Canada, Norway, Russia, Estonia. Group B included, in majority, specimens from France, Denmark, Norway, Russia, Japan, Sweden and St Pierre et Miquelon. Specimen n° 796, from Sweden, is found

divergent from group A and group B, and here named group C.

When fitting the phenotypes on the molecular tree (northern phenotype in black dots, Fig. 2 and 3), it appeared that 93% of the specimens of the northern phenotype belonged to group A, while 90% of the specimens of southern phenotype belong to group B. To avoid misinterpretation of the results, each of the five markers were re-extracted, re-amplified and re-sequenced for the nine specimens which did not fit the association between the northern phenotype and group A, or that between the southern phenotype and group B. The new sequences were identical to the first ones, confirming that our results were not biased by DNA contamination.

3.5 Analysis of the multigene dataset

3.5.1 Separate analysis

The results of the separate analysis of the five datasets are given in Table 5. The trees reconstructed from ITS1 and *Gap*C are presented in Figure 5. *Dicranum majus* was found to be monophyletic with *rbc*L only (PP = 0.89). The monophyly of group A received robust support from *rpl*32-*trn*L (PP = 0. 99) and that of group B was significantly supported by rbcL (PP = 1). The analysis showed that the mitochondrial marker does not vary, and one case of incongruence between chloroplastic and nuclear markers. Thus, topological incongruence at node III was found between ITS1 and *rpl*32-*trn*L.

3.5.2 Cytoplasmic dataset

Phylogenetic analysis were also conducted using a dataset combining the two chloroplastic and mitochondrial markers. The tree is presented in Figure 4. The analysis supported the monophyly of *Dicranum majus* (PP = 0.71), as well as that of group A and B, respectively PP = 0. 83 and PP = 1. The groups A and B appeared to share a sister-group relationship (PP = 0.75).

3.6 Blind identification test

The blinded phenotype identification of 8 specimens (Table 1) yielded controversial identifications of two samples from Canada (Québec, Saint Pierre and Miquelon Island), which were considered as intermediate between the northern or the southern phenotype.

3.7 Morphological results

The data matrix of the morphological characters are presented in Appendix II and Appendix III, and summarized in Figure 6 and Table 6. States "B" and "C" of characters 2, 3, and 9 were merged in the final matrix.

Among the twelve characters scored, the size of upper dorsal cell of the costa and the position of the lamella on the costa were found to be constant between the two groups, but leaf length is significantly smaller in group A than in group B (Wilcoxon-Mann-Whitney test, $p< 0.001$, Fig.7). None of the morphological characters were found to be exclusive to one group, except the upper leaf lamina, which is always smooth in members of group A. However, most representatives of group A tend to have (1) unistratose lamina, (2) dorsal cells in the lower part of the costa bigger than stereids, (3) unistratose lamina close to the margin, and (4) straight leaves. Most members of group B tend to have (1) dorsal cells in the lower part of the costa bigger than stereids, (2) bistratose guide cells, (3) width of the lumen of the lower leaf cells > 1/3 lamina width, (4) unistratose lamina close to the margin, (5) upper dorsal costa with lamella or projecting cell-ends, and (6) falcate leaves.

4. Discussion

4.1 Reliability of the results

4.1.1 Molecular results

Nuclear and cytoplasmic dataset were not entirely congruent. By comparing the support for individual nodes in the trees from the separate analysis, one conflict in delineating the two molecular groups was found (Table 5). The combination of the individual data sets to maximize the descriptive efficiency and explanatory power of the total information could be inadvisable when there is conflict (Miyamoto & Fitch, 1995), and Bull & *al*. (1993) advised to combine data sets only when they do not strongly support conflicting trees. In this study, we have chosen to do separate analysis, and then, to combine plastid and mitochondrion markers as they showed no incongruence. Thus, the conclusions drawn are based on the phylogenetic tree from the combined analysis; and more especially from the chloroplastic genome as the chondriom is not informative at this taxonomic level. The cause for the incongruence between the cytoplasmic and nrDNA topologies can not be identified with certainty. However, different causes from insufficient data, rapid diversification, horizontal gene transfer, hybridization, incomplete lineage sorting, convergence caused by natural selection, to variations in evolutionary rate, and methodological error can explained the observed topologies. In the current study, we discount poor sequence quality as a primary

source of error, as care was taken to resequence poor quality data and number critical specimens have multiple exemplars. Similarly, alignment cannot be an issue, as there are only a few short indels in ITS1 and GapC across *D. majus* that are not subject to alignment ambiguity.

Incomplete lineages sorting of ancestrally polymorphic lineages must be considered as a possible explanation for incongruence. In fact, after the initial divergence of species, a substantial amount of time is required before there will be a high probability of observing reciprocal monophyly at a sample of multiple loci (Hudson & Coyne, 2002). Thus, patterns of paraphyly across nuclear and cytoplasmic loci are predicted to be produced (Funk & Omland, 2003). Budding speciation (see Vanderpoorten & Long, 2006), the mechanism that we invoke as the potential cause to explain the formation of two groups within *D. majus*, further implies that the process is fairly recent and still in progress (Funk & Omland, 2003; Horandl & Stuessy, 2011 for review). However, to the degree that "parental" population exhibits geographical substructure is not enough known to conclude that a peripherical population is truely in a speciation process, as we have focused our study on European *D. majus* populations. These results must be confirmed by an increase of sampling in North America, Greenland, United-Kingdom and Ireland (type populations), Caucasus, Russia, China, and Taiwan (limit range in the South). However, because it reflects population history, this nested pattern is evolutionarily informative, allowing the polarization of the speciation event and of transition between traits (morphological patterns, geographical ranges, etc.) that accompany and may have promoted speciation (Funk & Omland, 2003).

4.1.2 Morphological vs molecular results

Our molecular study showed that the morphologically defined *D. majus* is composed of three molecular groups which one is composed of a single specimen from Sweden (n° 796). The occurrence of group C can be explained by different causes: divergent haplotype, DNA contamination, or misidentification. Poor sequence quality as a primary source of error can be discarded, as care was taken to resequence the critical sample. However, misidentification should be considered as the explanation of this topology. L.H., J.B. and A.P. identified again and separately the specimen and it come to conclusion that despite n° 796 looks like *D. majus* or possibly *D. flexicaule* in habit, several of the microscope characters (including the tong-like transverse section of the upper leaf) actually suggest *D. acutifolium*. The strange appearance of the plant could be explained by that it was collected in an odd place for *D. acutifolium*, a fen with pine trees, whereas the species is more likely to be found on Ca-rich

mountain heaths (4.1<pH<7.0) or on rocks (Dierssen, 2001). Thus, the present study showed that compared with angiosperms, the lack of a sufficient amount of morphological characters in bryophytes make it more difficult to identify specimens with confidence especially if samples were collected in "original" habitats.

Testing the taxonomic significance of morphological characters that have been used for delineation of the resulting phenotypes in *D. majus* showed no discriminatory characters but strong tendency (Table 6). Leaf length is the only character that consistently distinguishes the two groups A and B. Given our sampling, morphology alone is inadequate to circumscribe the two molecular groups. It is worth noting that, in mosses, classification has traditionally been based on a few characters in a context of complete lack of information regarding morphological evolution (Vanderpoorten & Jacquemart, 2004). Whether morphological variation in nature is genetically fixed or reflects environmental variation is particularly underestimated in *D. majus*, and more generally in genus *Dicranum*, and, it has long been acknowledged that a large part of morphological variation in many moss is due to plasticity (Vanderpoorten & Jacquemart, 2004, Hedenäs, 1996 for review). For instance, curvature of the costa, considered as a diagnostic feature of the phenotypes, is known to vanish under control conditions in *Amblystegium*, as well as leaf size characters significantly decreased after cultivation (Vanderpoorten & Jacquemart, 2004, Hedenäs, 1996). In the *Drepanocladus aduncus-polycarpus* complex, leaf curvature is correlated to the habitat moisture, falcate-leafed phenotypes growing in drier habitats (Hedenäs, 1996). The wide ecological amplitude of *D. majus* growing in both oceanic (e.g. Brittany) and arctic (e.g. Troms, Norway) type of climate (see Dierssen, 2001 and Fig.1) must make us careful on the weight we give to the characters discriminating the molecular groups. Greenhouse experiments should be helpful in assessing the origin of morphological variations (environmental or genetic), and delineating the groups.

4.2 Phylogeny and taxonomy of Dicranum majus

Dicranum majus forms a supported clade (PP = 0.71) from the branching leading to two molecular groups which are almost fitting the two phenotypes described by Hedenäs & *al*. (2006). The apparent "low" support of the clade *D. majus* can be attributed to the low number of outgroups, or that these outgroups are too distantly related to the ingroup, and/or the low level of molecular variation within the four markers. Thus, several studies have suggested that increasing the number of sampled taxa (here, number of outgroups) can enhance the accuracy of phylogenetic analysis (Flynns, & *al.*, 2005). Furthermore, Chen & *al.*

(2003) wonder whether a high bootstrap proportion should be given higher confidence than a lower one as it is often impossible to know from a single tree (supermatrix or supertree) whether the grouping are due to artefacts of phylogenetic reconstruction or due to common ancestry. The authors noted, however, that separate analysis provides other opportunities for assessing reliability. In the present study, monophyly of *D. majus* and its two subgroups are found more reliable in the separate analysis than in the combined analysis (Table 5). This congruence of inferences separately drawn from independent data is here considered as a good indicator of reliability of the combined analysis.

The adequation between the marker choice and the taxonomic level in the present intra-specific study must be considered as another possible explanation for the lower support of the monophyly of *D. majus* and its two molecular groups. In fact, some molecular markers can be inappropriate to resolve putative species because of little variation or low substitution rates (Vanderpoorten & Shaw, 2010). For example, Nad5-4 spacer (mtDNA) was found to perform poorly at the boundary between species and variety level (no parsimonious sites), in *D. majus*, confirming the lower variability of the mitochondrial genome compared to the plastid genome (Knoop & al., 2004), and its uselessness at this taxonomic level. However, monophyly of the species is confirmed as being robust in a broader molecular analysis of the genus *Dicranum* (Pichonet & *al*., in prep.).

The present molecular and morphological data almost fully support the existence of two well-separated groups in *D. majus* in Europe (Hedenäs & *al*, 2006), including a small northern type characterized by one row of guide cells and smooth lamina, and a southern type characterized by two row of guide cells and many lamina cells with spine-like dorsal projections. One specimen from Sweden did not fit either of the two molecular groups and two specimens from Canada were found morphologically intermediate. However, wide phenotypic plasticity is known to occur in the characters of moss gametophytes at the intraspecific level that is capable either of masking, or of suggesting erroneous genotypic differences (Touw, 1982), as is illustrated here by the results of the blind identifications (Table 1). We expected that a thorough sampling inside *D. majus*, with a focus on European populations, and use of molecular data would allow resolving the phylogenetic relationships of the northern and southern phenotypes as well as their taxonomic status. In this analysis, the monophyly of *D. majus* and the occurrence of two molecular groups were found robust. The two groups show a strong tendency for geographical segregation and morphological differentiation. Moreover, they occur in sympatry in southern Sweden, and northern America. In view of the existence of a few deviant accessions, we propose to treat the two groups as

geographical subspecies.

In addition, this study showed high phenotypic plasticity of *D. majus* in Europe (from S to N), North America and Asia. However, the usual morphological characters describing the species (e.g. Crum & Anderson, 1981; Bellolio-Trucco & Ireland, 1989)do not include its whole phenotypic plasticity. A new morphological circumscription of *D. majus* is needed, covering its entire range of variation. Ideally, this should be done within a robust phylogenetic framework including its close relatives (*D. scoparium, D. polysetum, D. bonjeanii, D. leioneuron, D. acutifolium)* in order to avoid misidentification or misinterpretation. The genetic relationships found in the present study constitute a basis for future investigations into the morphological and genetic variation of *D. majus* within its entire range. Further sampling from the rest of the range of *D. majus* (e.g. West Canada, China, Taiwan, Mongolia, East of Europe) and the use of more variable markers (i.g. microsatellites) may help us to better understand the relationships and biogeography of the subspecies of *D. majus*.

5. Taxonomic conclusions

Among the six varieties currently recognized in *D. majus*, var. *minus*, var. *condensatum*, and var. *capnodes* possibly refer to the northern phenotype based on their descriptions. Study of the types should confirm their status. Among the remaining ones, *D. majus* var. *orthophyllum* is known to belong to the southern phenotype (Hedenäs & *al*., 2006), but the status of the varieties with more or less undulate leaves (*D. majus* var. *subundulatum*, and *D. majus* var. *undulascens*) is unclear and type material has to be studied. Possibly, these two varieties belong to other species.

Key to the subspecies of *Dicranum majus* Turn. resulting from this study

1. Leaves to 1.5 cm long, falcate, upper portion of dorsal lamina rough and with a bistratose submarginal band (cross section)….....1. *D. majus* subsp. *majus*

2. Leaves to 1 cm long, almost straight, upper portion of lamina smooth, unistratose…….....…2. *D. majus* subsp. *hedenaesii*

1. ***Dicranum majus* Turn.** (Muscologiae Hibernicae Spicilegium: 59. 1804) **subsp. *majus*** Fig. 2A

= *Dicranum majus* var *orthophyllum* A. Braun ex. Milde, Bryologia Silesiaca: 71. 1869.

Leaves distinctly falcate, 0.45-1.5 cm long; upper margin denticulate; lamina (transverse section) spinose above, with a bistratose band (cross section); costa with two layers of guide cells.

Distribution: South and Central Europe, North America, Asia.

Specimens examined: see Appendix I.

2. ***Dicranum majus* subsp. *hedenaesii* Pichonet, subsp. nov.** Fig. 2B

? = *Dicranum majus* var. *minus* Bruch & Schimp., London Journal of Botany 2: 666. 1843 (Type not seen).

? = *Dicranum majus* var. *condensatum* I. Hagen, Det K. Norske Videnskabers Selskabs Skrifter 1914 (1): 157 (Type not seen).

? = *Dicranum majus* var. *capnodes* I. Hagen, Det K. Norske Videnskabers Selskabs Skrifter 1914 (1): 161 (Type not seen).

Type: Sweden. Torne Lappmark, Karesuando, along Merasjoki from 1 km above to 1 km below the inflow of the brook Nittyjoki (30L 2j), Moist pit, 22, July, 1990, L. Hedenäs, M. Aronsson, **B6158** (holotype: S).

Folia erecta vel subfalcata, usque ad 1 cm longa, margine foliorum integra vel obsolete denticulata.

Leaves erect to slightly falcate, 0.4-1.0 cm long; upper margin slightly denticulate to entire; lamina smooth, unistratose (cross section); costa with one layer of guide cells.

Distribution: Northern Europe, northern North America.

Specimens examined: see Appendix I.

The subspecies is dedicated to L. Hedenäs who contributed to its discovery by its morphological study (2006), and who kindly provided the material from Sweden and Norway.

Acknowledgements

We would like to thanks all the people who provided plant material; these include Jean Faubert, Serge Müller, Simon Laegaard, and Kai Vellak. Thanks are also due to the directors/curators of the following herbaria for the loan of herbarium specimens and permission to take tissue samples: S (Lars Hedenäs), MW (Michael Ignatov), (Masanobu Higushi). We are grateful to Philippe Mennecier for translating the Russian Moss Flora, Denis Lamy for historical information on taxa description, and Amandine Allard for assistance in checking and digitalizing the *Dicranum* types in herbarium PC. We also thank Regis Debruyne for helpful comments on the manuscript

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Tables

Table 1: Blind-test identification of selected specimens with their membership to the respective molecular groups

Table 2: Characters investigated for twelve specimens of group A and group B, ($^{\Delta}$) modified from Hedenäs & *al. (*2006); (*) new character. For each specimen ten lower cross sections, ten upper cross sections, and five entire youth but mature leaves were observed

Table 3: Primers used to amplify the specimens of this study

Table 4: Numbers of nucleotide sites included in the analysis, variable sites, and parsimony-informative sites for each locus used in the phylogenetic analysis of *D. majus*

Table 5: Node robustness, PP: posterior probability; X: node not found, and a conflicting hypothesis is supporting by PP>0.75; -: node not found, and there is no robust hypothesis, PP<0.75

Table 6: Percentage of occurrence of state of characters cited in Table 2, in both molecular groups

Figures

Figure 1: Population sampling: (yellow circles) southern phenotype, (black circles) northern phenotype according to Hedenäs & *al.* (2006); (green) distributional range of *Dicranum majus* compiled from Worley & Iwatsuki (1970), Sergio & Carvalho (2003), Nyholm (1954), Natcheva & Ganeva (2005), Krüschner & Erdau (2005), Jensen (1939), Ignatov & *al.* (2006), Iwatsuki (2004), Ireland & *al.* (1987), Higuchi & Nishimura (2003) Gao & *al.* (1999), Colacino & Sabovlejic (2006), Bergþór (2003), Anderson & *al.* (1990)

Figure 2: (from bottom to top) structure of the costa in the lower leaf, middle leaf, upper leaf **(A)** *Dicranum majus* subspecies *majus*, (B) *Dicranum majus* subspecies *hedenassii*, (C) controversial specimens

Figure 3: Intron-Exon structure of *Gap*C based on the alignment of NCBI sequences

Figure 4: The signal from combined cytoplasmic markers: *rpl*32-*trn*L, *rbc*L and *Nad*5-4, (black dots) northern phenotype specimens

Figure 5: The signal of nuclear markers *Gap*C and ITS1, (black dots) Northern phenotype

Figure 6: State of characters investigated for ten cross sections, in order of appearance in Table 2; (empty square) state of character A in the ten cross sections, (black square) state of character B in the ten cross sections, (bicolour square) state of character A and B in the ten cross sections, (nr) no information available

Figure 7: Leaf length box plots of (green) group A and (red) group B

Appendix

Appendix I: Citation of vouchers (subsp. *majus*, subsp. *hedenaesii*, intermediate forms).

Appendix II: Data set matrix of the morphological characters observed in ten cross section leaves for twelve specimens in each molecular group chosen at random

Appendix III: Data set matrix of the morphological characters observed in five entire leaves for twelve specimens in each molecular group chosen at random

Figure 1

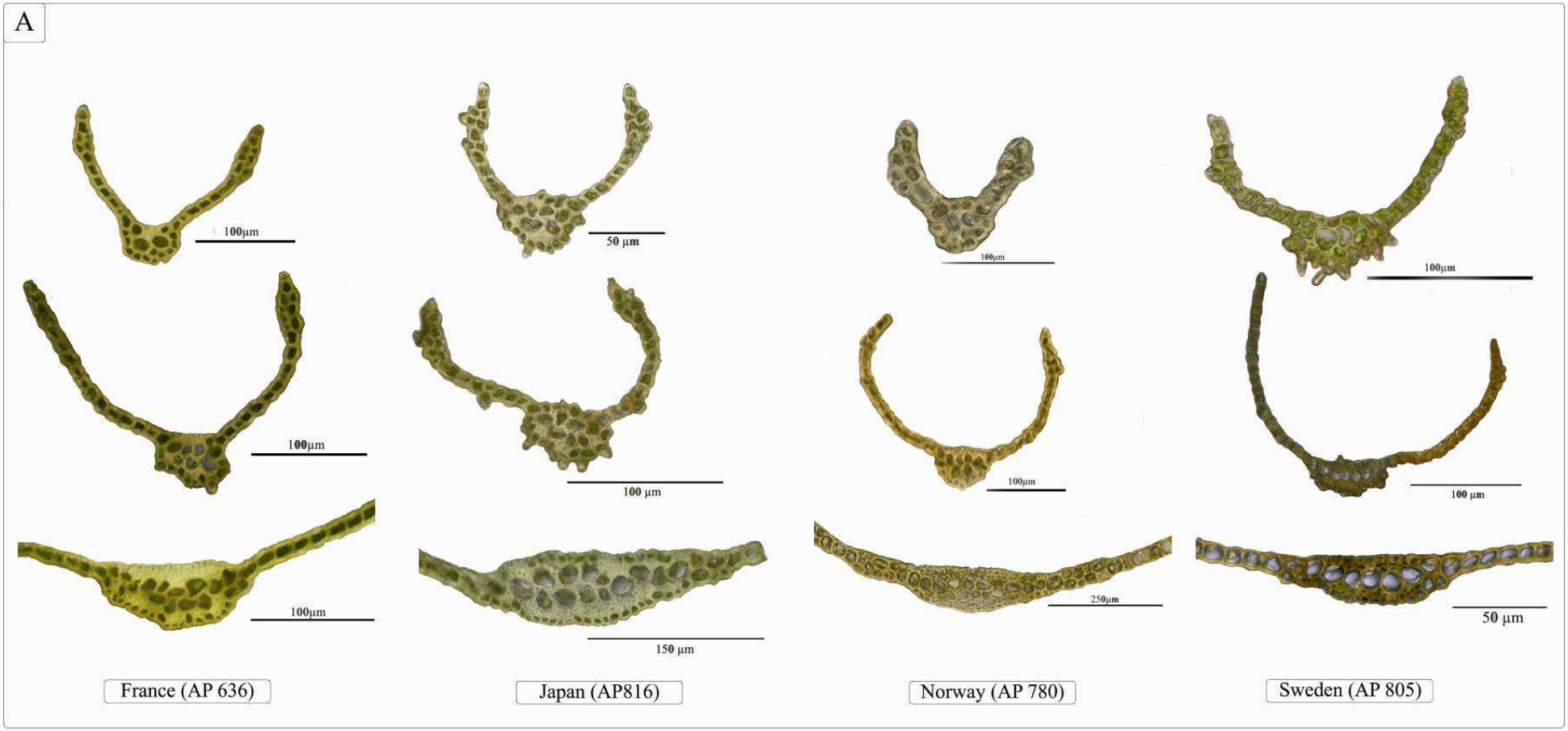

A
100µm
50 µm
100µm
100µm
100 µm
100µm
100µm
100 µm
100µm
250µm
50 µm
150 µm
France (AP 636)
Japan (AP816)
Norway (AP 780)
Sweden (AP 805)

Figure 2A

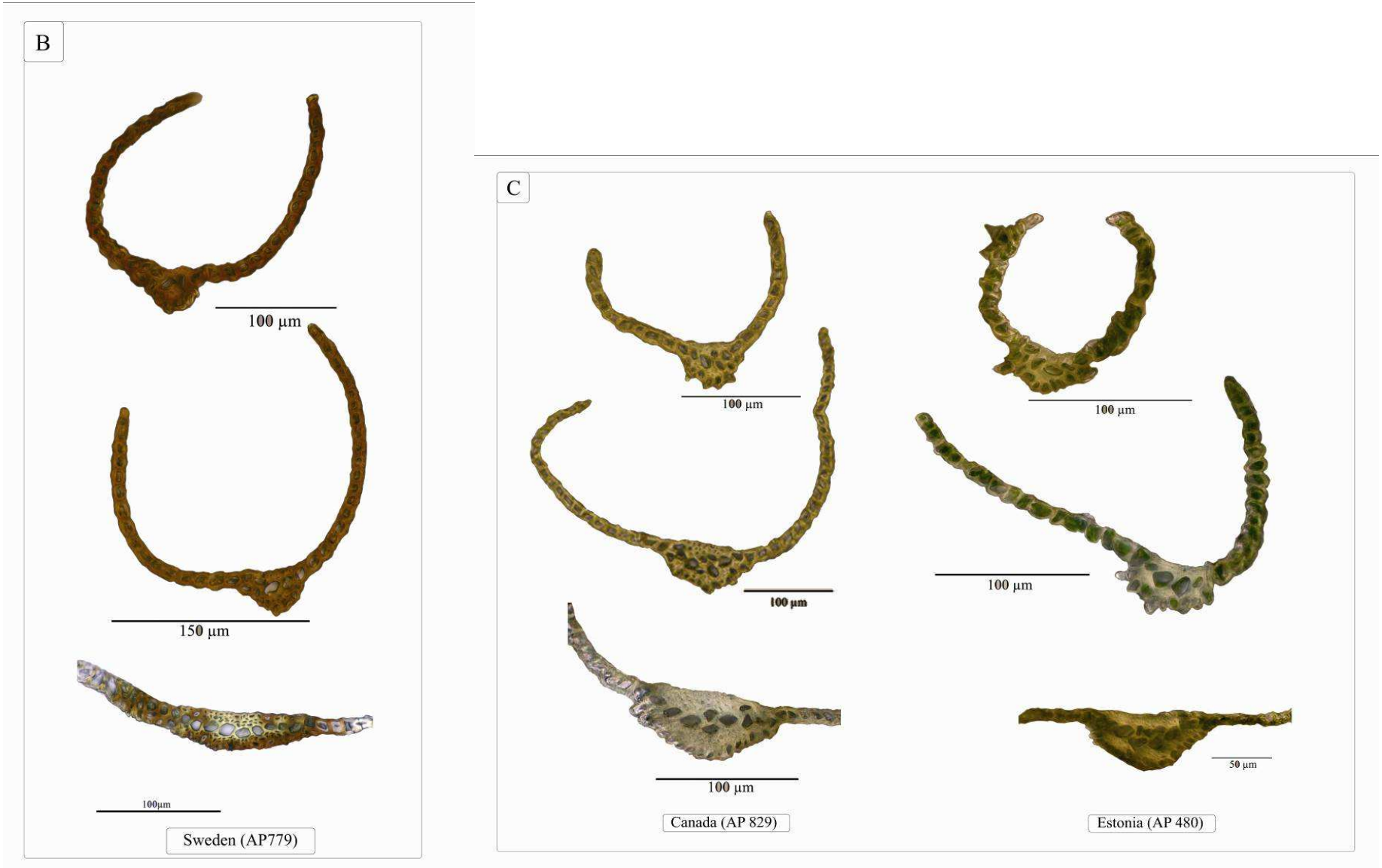

B
100 µm
150 µm
100µm
Sweden (AP779)
C
100 µm
100 µm
100 µm
100 µm
100 µm
50 µm
Canada (AP 829)
Estonia (AP 480)

Figure 2B-2C

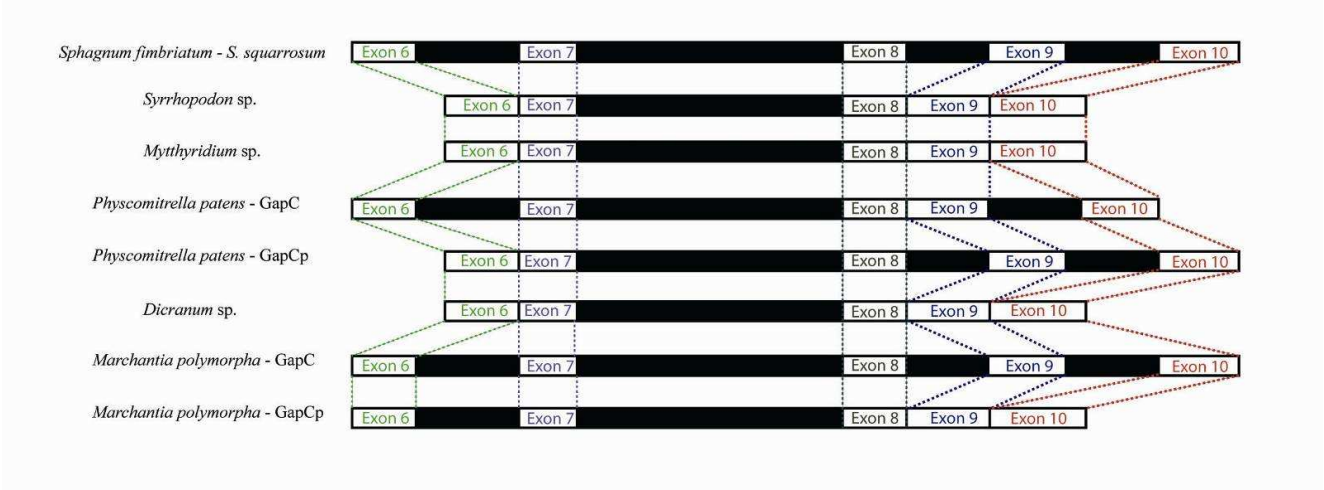

Sphagnum fimbriatum - S. squarrosum
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
Syrrhopodon sp.
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
Mytthyridium sp.
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
Physcomitrella patens - GapC
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
Physcomitrella patens - GapCp
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
Dicranum sp.
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
Marchantia polymorpha - GapC
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10
Marchantia polymorpha - GapCp
Exon 6
Exon 7
Exon 8
Exon 9
Exon 10

Figure 3

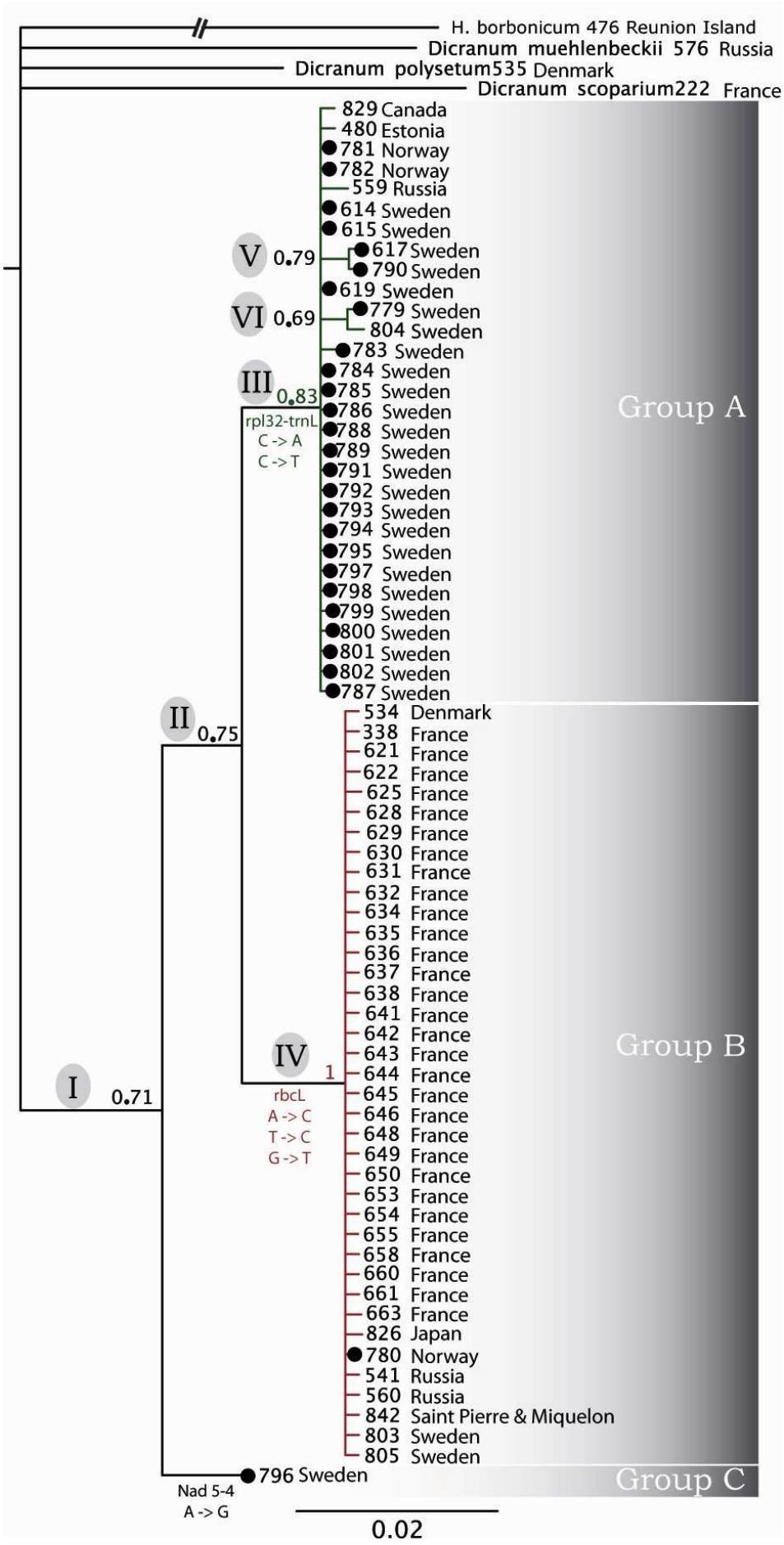
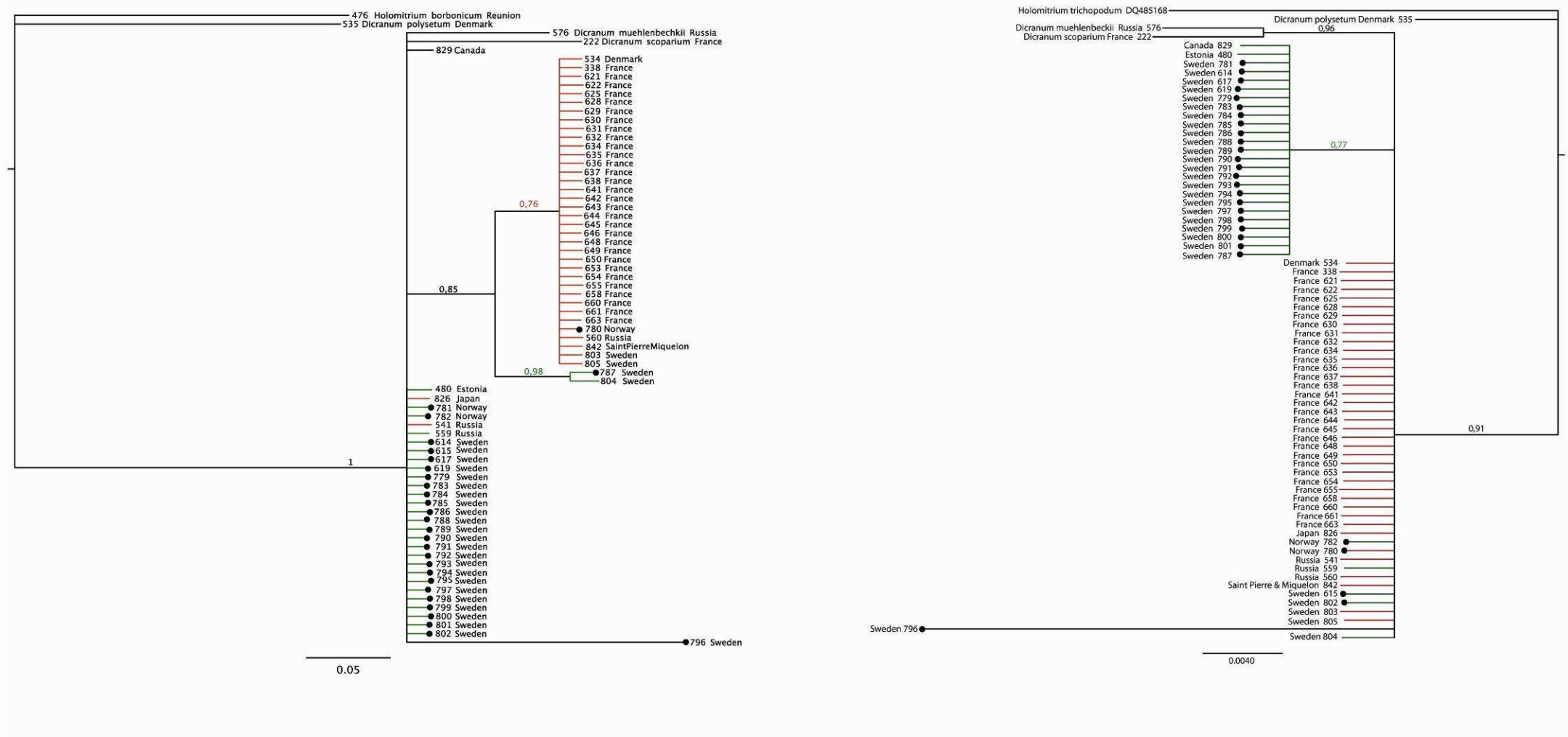

H. borbonicum 476 Reunion Island
Dicranum muehlenbeckii 576 Russia
Dicranum polysetum535 Denmark
Dicranum scoparium222 France
829 Canada
480 Estonia
781 Norway
782 Norway
559 Russia
614 Sweden
615 Sweden
V 0.79
617 Sweden
790 Sweden
619 Sweden
VI 0.69
779 Sweden
804 Sweden
783 Sweden
784 Sweden
III 0.83
785 Sweden
786 Sweden
788 Sweden
789 Sweden
791 Sweden
792 Sweden
793 Sweden
794 Sweden
795 Sweden
797 Sweden
798 Sweden
799 Sweden
800 Sweden
801 Sweden
802 Sweden
787 Sweden
Group A
rpl32-trnL
C -> A
C -> T
II 0.75
534 Denmark
338 France
621 France
622 France
625 France
628 France
629 France
630 France
631 France
632 France
634 France
635 France
636 France
637 France
638 France
641 France
642 France
643 France
644 France
645 France
646 France
648 France
649 France
650 France
653 France
654 France
655 France
658 France
660 France
661 France
663 France
826 Japan
780 Norway
541 Russia
560 Russia
842 Saint Pierre & Miquelon
803 Sweden
805 Sweden
Group B
IV
1
rbcL
A -> C
T -> C
G -> T
I 0.71
796 Sweden
Group C
Nad 5-4
A -> G
0.02

Figure 4


476 Holomitrium borbonicum Reunion
535 Dicranum polysetum Denmark
576 Dicranum muehlenbechkii Russia
222 Dicranum scoparium France
829 Canada
534 Denmark
338 France
621 France
622 France
625 France
628 France
629 France
630 France
631 France
632 France
634 France
635 France
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654 France
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658 France
660 France
661 France
663 France
780 Norway
560 Russia
842 SaintPierreMiquelon
803 Sweden
805 Sweden
787 Sweden
804 Sweden
480 Estonia
826 Japan
781 Norway
782 Norway
541 Russia
559 Russia
614 Sweden
615 Sweden
617 Sweden
619 Sweden
779 Sweden
783 Sweden
784 Sweden
785 Sweden
786 Sweden
788 Sweden
789 Sweden
790 Sweden
791 Sweden
792 Sweden
793 Sweden
794 Sweden
795 Sweden
797 Sweden
798 Sweden
799 Sweden
800 Sweden
801 Sweden
802 Sweden
796 Sweden
0,76
0,85
0,98
1
0.05
Holomitrium trichopodum DQ485168
Dicranum polysetum Denmark 535
Dicranum muehlenbeckii Russia 576
Dicranum scoparium France 222
0,96
Canada 829
Estonia 480
Sweden 781
Sweden 614
Sweden 617
Sweden 619
Sweden 779
Sweden 783
Sweden 784
Sweden 785
Sweden 786
Sweden 788
Sweden 789
Sweden 790
Sweden 791
Sweden 792
Sweden 793
Sweden 794
Sweden 795
Sweden 797
Sweden 798
Sweden 799
Sweden 800
Sweden 801
Sweden 787
0,77
Denmark 534
France 338
France 621
France 622
France 625
France 628
France 629
France 630
France 631
France 632
France 634
France 635
France 636
France 637
France 638
France 641
France 642
France 643
France 644
France 645
France 646
France 648
France 649
France 650
France 653
France 654
France 655
France 658
France 660
France 661
France 663
Japan 826
Norway 782
Norway 780
Russia 541
Russia 559
Russia 560
Saint Pierre & Miquelon 842
Sweden 615
Sweden 802
Sweden 803
Sweden 805
Sweden 804
0,91
Sweden 796
0.0040

Figure 5

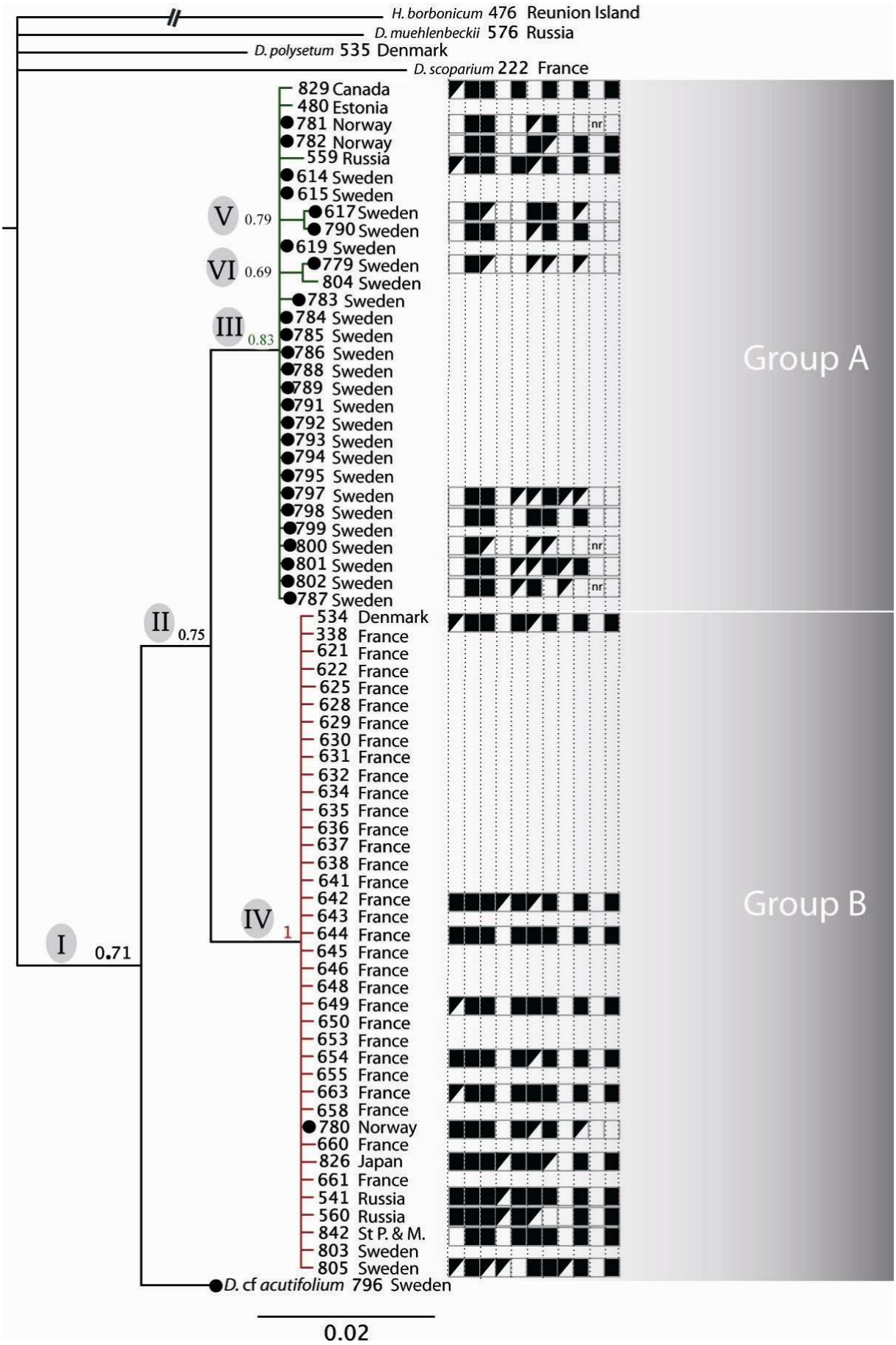

H. borbonicum 476 Reunion Island
D. muehlenbeckii 576 Russia
D. polysetum 535 Denmark
D. scoparium 222 France
829 Canada
480 Estonia
781 Norway
782 Norway
559 Russia
614 Sweden
615 Sweden
V 0.79
617 Sweden
790 Sweden
619 Sweden
VI 0.69
779 Sweden
804 Sweden
783 Sweden
784 Sweden
III 0.83
785 Sweden
786 Sweden
788 Sweden
789 Sweden
791 Sweden
792 Sweden
793 Sweden
794 Sweden
795 Sweden
797 Sweden
798 Sweden
799 Sweden
800 Sweden
801 Sweden
802 Sweden
787 Sweden
II 0.75
534 Denmark
338 France
621 France
622 France
625 France
628 France
629 France
630 France
631 France
632 France
634 France
635 France
636 France
637 France
638 France
641 France
642 France
643 France
IV 1
644 France
645 France
646 France
648 France
649 France
650 France
653 France
654 France
655 France
663 France
658 France
780 Norway
660 France
826 Japan
661 France
541 Russia
560 Russia
842 St P. & M.
803 Sweden
805 Sweden
I 0.71
D. cf acutifolium 796 Sweden
0.02
Group A
Group B
nr

Figure 6

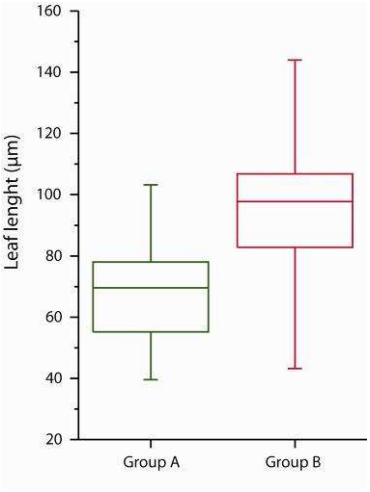


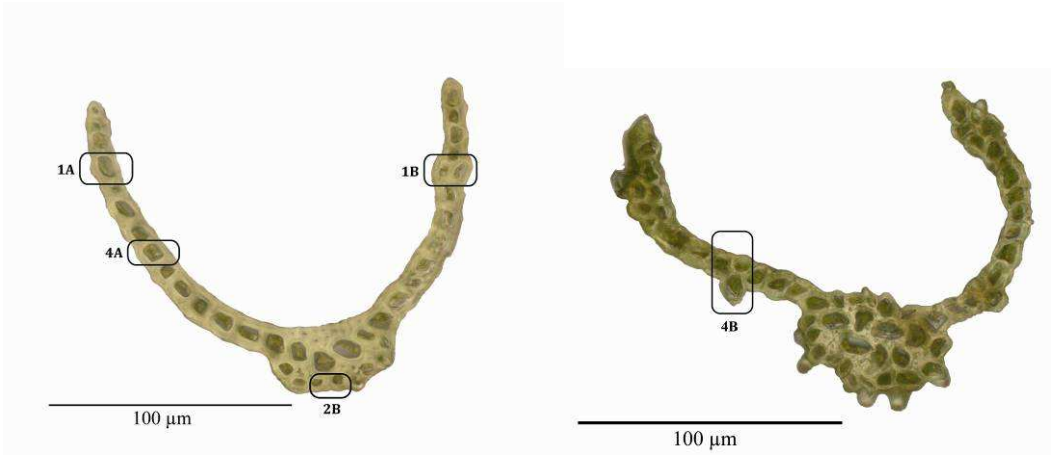
Figure 7

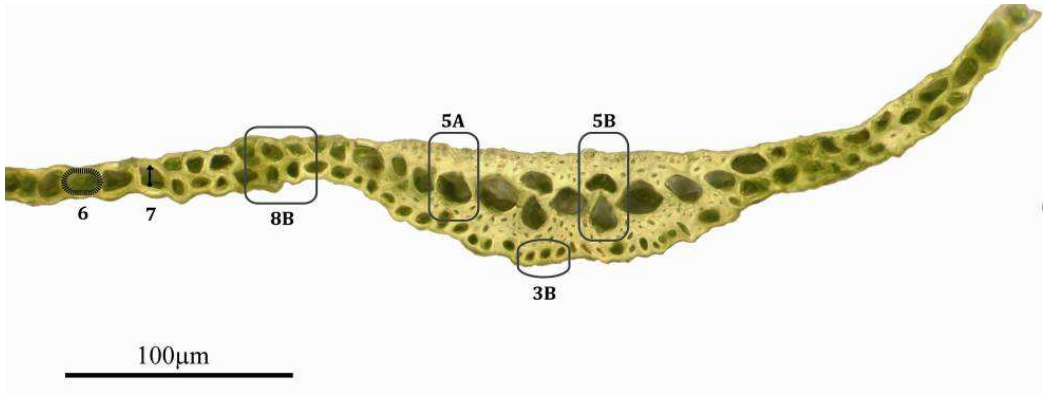
Table 1

LOCALITY	Specimen number	Det. A. P.	Det. L. H.	Det. J. B.	Molecular group
Japan	816	S	S	S	B
Russia	560	S	S	S	B
Russia	541	S	S	S	B
Russia	559	S	S	S	A
St Pierre & Miquelon	538	S	N	S	B
Canada	492	S	intermediate	S	A
Estonia	480	S	S	S	A
Sweden	796	cf. acutifolium	cf. acutifolium	cf. acutifolium	C

Table 2

1$^{\Lambda}$	Upper lamina cells close to the margin: A) unistratose, B) bistratose; if B) number of bistratose layers
2*	Dorsal cells in **upper** third of costa: A) as big as stereids, B) bigger than stereids but of an homogeneous shape, C) bigger than stereids but of two shapes
3*	Dorsal cells in **lower** third of costa: A) as big as stereids, B) bigger than stereids but of an homogeneous shape, C) bigger than stereids but of two shapes
4$^{\Lambda}$	Upper dorsal leaf lamina cells: A) smooth, B)with projecting cell-ends; if B) number of projecting cell-ends
5$^{\Lambda}$	Guide cells in lower third of costa: A) unistratose, B) bistratose; if B) number of guide cells in layer one and layer two
6*	Shape of lamina cells of lower leaf (transverse section): A) isodiametric, B) rectangular
7*	Lumen cells of lower leaf (transverse section): ≤ 1/3 lamina width, B) ≥ 1/3 lamina width
8*	Lamina cell layers of lower leaf close to the costa : A) unistratose, B) bistratose
9*	Upper dorsal costa roughness: A) smooth, B) with lamella, C) with papilla; if B) number of lamella





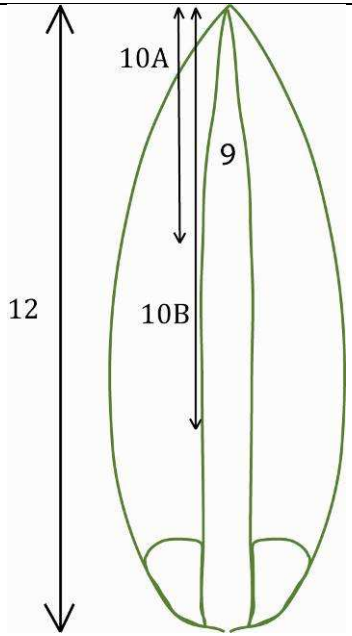
10 Upper dorsal costa roughness: A) lamella in upper third of costa, B) lamella in upper two-third of costa Δ	
11 Leaf curvature (3 to 5 leaves observed): A) straight, B) falcate Δ	
12 Leaf length (µm, 3 to 5 leaves observed) Δ	

Table 3

Genome	Genomic region	Primer name	Sequence (5'-3')	Reference
chloroplast	*rbc*L part 1	rbcL-TKT-F1 (F1_F)	ACC-CAW-GTC-ACC-ACA-AAC-RGA-G	Ebihara & *al.* (2007)
		TKT-F1-TKT-2PRN (2PRN-R)	CGT-TCT-CCT-TCC-AGT-TTR-CCT-ACT-ACA-GT	Ebihara & *al.* (2007)
		rbcL_300S	AAA-GCT-TTA-CGA-GCC-TTG-CGT-CTA-GAA	
		rbcL_400AS	ATC-CCA-ATA-ATG-GAC-GAC-CAT-ATT-TG	
		rbcL_600S	TCC-CAA-GCT-GAA-ACA-GGT-GAA-ATT-A	
		rbcL_800AS	TCA-ATA-ACT-GCA-TGC-ATT-GCA-CGG-T	
	*rpl*32-*trn*L$^{(UGA)}$	rpl32	ACC-CAW-GTC-ACC-ACA-AAC-RGA-G	Shaw & *al.* (2007)
		trnL	CGT-TCT-CCT-TCC-AGT-TTR-CCT-ACT-ACA-GT	Shaw & *al.* (2007)
		R32_200S	TAT-ATA-GCA-CCT-AAC-AAT-TAA-ATG-T	
		R32_500AS	TCA-ATT-TTA-ATG-GGG-CAT-AGC-GTA-G	
mitochondria	*Nad* 5-4	Nad5-4S	GGA-ATT-TCG-TAC-ACA-TTT-CG	
		Nad5-4AS	AAT-ACT-TCG-TAT-CAG-TCG-TA	
		Nad5-4_300S	AGC-CTA-AGC-TAT-GCT-TTG-CGG-TCT-A	
		Nad5-4_400AS	ATC-ATT-CCA-TCA-TTC-CAA-CCT-TTA	
		Nad5-4_600S	TAG-TAT-GCG-CGT-ATA-AAA-GCT-CAT-C	
		Nad5-4_750AS	ACT-CTT-TTT-CCA-AAA-GAT-CCC-GAG-GA	
nuclear	*Gap*C	GapC_E7S	TGC-CAA-GGT-TAT-CAA-CGA-CAA-GTT-CG	
		GapC_E10AS	CGG-TGT-AAC-CCA-ARA-TRC-CCT-TCA-TC	
	ITS 1	Its5-bryo	GGA-AGG-AGA-AGT-CGT-AAC-AAG-G	Sabovljevic & *al.* (2005)
		ItsC-bryo	GCA-ATT-CAC-ACT-ACG-TAT-CGC	Sabovljevic & *al.* (2005)

Table 4

	Amplification lenght	Alignment length	Amplification success (%)	Number of variable sites	Number of parsimonious sites	Percentage of parsimonious sites
ITS1 (nu)	322-376	388	96	17	2	0.52
*rbc*L (cp)	773-791	783	99	26	13	1.66
*rpl*32-*trn*L (cp)	524-543	543	96	43	15	2.76
Nad 5-4 (mt)	758-759	763	96	8	0	0.00
*Gap*C (nu)	416-470	498	100	27	11	2.21

Table 5

	Cytoplasmic DNA	Chloroplastic markers		Mitochondrial marker	Nuclear markers	
		***rbc*L**	***rpl*32-*trn*L**	***Nad*5-4**	**ITS1**	***Gap*C**
Reliable nodes	PP	PP	PP	PP	PP	PP
I	0.71	0.89	-	-	-	-
II	0.75	-	-	-	-	-
III	0.83	-	0.99	-	X	-
IV	1.00	1.00	-	-	-	-
V	0.79	0.94	-	-	-	-
VI	0.69	0.74	-	-	-	-

Table 6

State of character		Group A (%)	Group B (%)
1	**A**	83	9
	B	-	58
	A/B	17	33
2	**A**	-	-
	B	100	100
	A/B	-	-
3	**A**	-	-
	B	75	92
	A/B	25	8
4	**A**	100	58
	B	-	-
	A/B	-	42
	A	58	8

5	**B**	17	92
	A/B	25	-
6	**A**	8	-
	B	34	58
	A/B	58	42
7	**A**	8	8
	B	67	84
	A/B	25	8
8	**A**	75	92
	B	-	-
	A/B	25	8
9	**A**	25	
	B	50	92
	A/B	25	8
10	**A**	100	100
	B	-	-
	A/B	-	-
11	**A**	75	8
	B	25	92
	A/B	-	-

Appendix I

DNA voucher	N° study	Hb voucher	Latitude	Longitude	Specimen examined
AP779	DMa-SE01	B6158 (S)	68°26'24.18"N	22°28'15.33" E	subsp. *hedenaesii*
AP780	DMa-SE02	B63133 (S)	70°27'N	23°24'E	subsp. *majus*
AP781	DMa-SE03	B63137 (S)	70°28'N	23°24'E	subsp. *hedenaesii*
AP782	DMa-SE04	B82830 (S)	69°37'N	20°15'E	subsp. *hedenaesii*
AP783	DMa-SE05	B100386 (S)	64°32'N	15°38'E	no obs.
AP784	DMa-SE06	B115134 (S)	66°21'N	17°03'E	no obs.
AP785	DMa-SE07	B131032 (S)	64°35'N	16°37'E	no obs.
AP786	DMa-SE08	B131036 (S)	66°07'45.88"N	17°09'25.5 1"E	no obs.
AP787	DMa-SE09	B131049 (S)	67°32'21.56"N	20°06'44.3 3"E	no obs.
AP788	DMa-SE10	B131050 (S)	67.1667	23.1667	no obs.
AP789	DMa-SE11	B131054 (S)	67.1667	23.1667	no obs.
AP790	DMa-SE12	B131057 (S)	67°29'23.69"N	22°39'37.4 5"E	subsp. *hedenaesii*
AP791	DMa-SE13	B131058 (S)	67°29'23.69"N	22°39'37.4 5"E	no obs.
AP792	DMa-SE14	B131059 (S)	67°40'33.36N	21°38'30.83 "E	no obs.
AP793	DMa-SE15	B131063 (S)	68°05'43.64"N	20°44'22.9 2"E	no obs.
AP794	DMa-SE16	B131069 (S)	68°05'43.64"N	20°44'22.9 2"E	no obs.
AP795	DMa-SE17	B131071 (S)	67°38'48.70"N	21°03'13.2 4"E	no obs.
AP796	DMa-SE18	B131073 (S)	67°12'45.60"N	23°22'00.9 4"E	cf. *D. acutifolium*
AP797	DMa-SE19	B131077 (S)	66°57'35.96"N	19°49'14.1 8"E	subsp. *hedenaesii*
AP798	DMa-SE20	B131078 (S)	66°36'18.58"N	19°50'09.0 5"E	subsp. *hedenaesii*
AP799	DMa-SE21	B131083 (S)	66°25'49.30"N	19°41'07.6 3"E	no obs.
AP800	DMa-SE22	B167305 (S)	67°43'54.48"N	22°49'13.2 2E	subsp. *hedenaesii*
AP801	DMa-SE23	B167307 (S)	64°24'21.40"N	18°30'07.5 5"E	subsp. *hedenaesii*
AP802	DMa-SE24	B167308 (S)	63°10'16.29"N	14°57'33.0 5"E	subsp. *hedenaesii*
AP614	DMa-SE30	B131066 (S)	68°05'27.84"N	21°41'49.7 1"E	no obs.
AP615	DMa-SE26	B131048 (S)	67°08'02.27"N	20°39'39.5 1"E	no obs.
AP617	DMa-SE29	B131064 (S)	67°43'48.20"N	21°59'57.2 7"E	subsp. *hedenaesii*
AP619	DMa-SE25	B114445 (S)	65°42'N	18°45'E	no obs.
AP803	DMa-SE31	B88574 (S)	59°02'01.31"N	16°45'05.50 "E	no obs.
AP804	DMa-SE32	B143466 (S)	61°35'34"N	15°17'53"E	no obs.
AP805	DMa-SE33	B160335 (S)	58°56'N	18°18'E	subsp. *majus*
AP338	DMa-FR01				no obs.
AP621	DMa-FR03	DMa-FR03			no obs.
AP622	DMa-FR04	AU7020			no obs.
AP625	DMa-FR05	DMa-YveliNes 01	48°40'22.79"N	1°58'17.98"E	no obs.
AP628	DMa-FR06	Bg-06	N48°19,771	W03°48,578	no obs.
AP629	DMa-FR07	Bg-04	N48°20,477	W03°23,107	no obs.
AP630	DMa-FR08	Bg-03	N48°20,484'	w 03°23,117	no obs .
AP631	DMa-FR09	Bg-05	N48°19,777	W03°48,579	no obs.
AP632	DMa-FR10	Bg-07	N48°19,771	W03°48,578	no obs.
AP634	DMa-FR11	Bg-02	N48°20,481	w03°23,103	no obs.
AP635	DMa-FR12	Bg10			no obs.
AP636	DMa-FR13	Bg11			no obs.
AP637	DMa-FR14	Bg12			no obs.
AP638	DMa-FR15	Bg13			no obs.
AP641	DMa-FR16	Bg-40	N49°25,001	E00°41,362	no obs.
AP642	DMa-FR17	Bg-25	N48°06,025	W04°10,615	subsp. *majus*
AP643	DMa-FR18	Bg-22	N°48°05,957	W04°10,669	no obs.

AP644	DMa-FR19	Bg-24	N48°05,992	W04°10,644	subsp. *majus*
AP645	DMa-FR20	Bg-23	N°48°05,957	W04°10,669	no obs.
AP646	DMa-FR21	Bg-26	N48°06,020	W04°10,601	no obs.
AP648	DMa-FR22	Bg18			no obs.
AP649	DMa-FR23	Bg-20	N48°22;239	W03°42,867	subsp. *majus*
AP650	DMa-FR24	Bg-21	N48°22;239	W03°42,867	no obs.
AP653	DMa-FR25	Bg-27	N48°01,846	W04°02,972	no obs.
AP654	DMa-FR26	Bg-33	N47°50,132	W03°33,095	subsp. *majus*
AP655	DMa-FR27	Bg-19	N48°22,249	W03°42,890	no obs.
AP658	DMa-FR28	Bg-34	N47°50,210	W03°33,051	no obs.
AP660	DMa-FR29	Bg-35	N47°50,210	W03°33,051	no obs.
AP661	DMa-FR30	Bg-29	N48°01,848	W04°02,959	no obs.
AP663	DMa-FR31				subsp. *majus*
AP816, AP 826	DMa_JP01	49244 (TNS)	36°05'27.98"N	138°19'46.35"E	subsp. *majus*
AP541	DMa-RU01	06-3324 (MW)	43°20'53.40"N	133°34'12.47"E	subsp. *majus*
AP559	DMa-RU02	(MW)	59°53'34.99"N	38°41'36.15"E	no obs.
AP560	DMa-RU03	06-1316 (MW)	44°11'49.12"N	145°57'27.32"E	subsp. *majus*
AP480	DMa-EE01		59°34'57"N	26°15'01"E	intermediate form
AP534	DMa-DK01		56°02'10.70"N	12°36'48.64"E	subsp. *majus*
AP492, AP829	DMa-CA01	63	48°18,335'N	068°38,734'W	intermediate form
AP538, AP 842	DMa-PM01				subsp. *majus*

Appendix II

		Character number	1	2	3	4	5	5	6	7	8
Molecular group	**Country**	**Specimen number**	**Upper lamina cells close to the margin**	**Dorsal cells of costa UPPER Leaf**	**Dorsal cells of costa LOWER Leaf**	**Upper dorsal leaf lamina cells**	**Nb guide cells in lower third of the costa**	**Nb of guide cells in layer two**	**Shape of lamina cells of lower leaf in transverse section**	**Lumen cells of lower leaf in transverse section**	**Lamina cells layers close to the costa**
A	**Sweden**	**617.1**	A	B	C	A	B5	A	B	B	A
		617.2	A	B	B	A	B5	A	B	B	A
		617.3	A	B	B	A	B5	A	B	B	A
		617.4	A	B	A	A	B7	A	B	B	A
		617.5	A	B	B	A	B5	A	B	B	A
		617.6	A	B	B	A	B5	A	B	B	A
		617.7	A	B	A	A	B5	A	B	B	A
		617.8	A	B	B	A	B5	A	B	B	A
		617.9	A	B	A	A	B8	A	B	B	A
		617.10	A	B	B	A	B5	A	B	B	A
B	**France**	**644.1**	B4	B	B	A	B4	B	B	B	A
		644.2	B1	B	B	A	B4	B	B	B	A
		644.3	B3	B	B	A	B4	B	B	B	A
		644.4	B4	B	B	A	B4	B	B	B	A
		644.5	B6	B	B	A	B4	B	B	B	A
		644.6	B2	B	B	A	B7	B	B	B	A
		644.7	B4	B	B	A	B4	B	B	B	A
		644.8	B1	B	B	A	B6	B	B	B	A
		644.9	B4	B	B	A	B5	B	B	B	A
		644.10	B5	B	B	A	B5	B	B	B	A
B	**Japan**	**816.1**	B8	B	B	B1	B9	C	B	B	A
		816.2	B4	B	B	A	B6	C	B	B	A
		816.3	B8	B	B	A	B7	C	B	B	A
		816.4	B5	B	B	A	B7	C	B	B	A
		816.5	B6	B	B	A	B7	C	B	B	A
		816.6	B10	B	B	A	B7	C	B	B	A
		816.7	B6	B	B	A	B6	C	B	B	A
		816.8	B9	B	B	A	B9	C	B	B	A
		816.9	B6	B	B	A	B10	C	B	A	A
		816.10	B6	B	B	A	B9	C	B	B	A
A	**Western Russia**	**559.1**	A	B	B	A	B6	C	B	B	A
		559.2	A	B	B	A	B7	C	B	B	A
		559.3	B3	B	B	A	B7	C	A	B	A
		559.4	A	B	B	A	B8	C	B	B	A
		559.5	A	B	B	A	B5	C	B	B	A
		559.6	B2	B	B	A	B8	C	B	B	A
		559.7	B2	B	B	A	B7	C	B	B	A
		559.8	A	B	B	A	B9	C	B	B	A
		559.9	B1	B	B	A	B5	B	B	B	A
		559.10	A	B	B	A	B6	C	B	B	A
		492.1	A	B	B	A	B7	B	A	B	A

		492.2	A	B	B	A	B9	B	A	B	A
		492.3	A	B	B	A	B8	B	A	B	A
		492.4	A	B	B	A	B7	B	A	B	A
A	**Canada**	**492.5**	A	B	B	A	B7	B	A	B	A
		492.6	A	B	B	A	B7	C	A	B	A
		492.7	A	B	B	A	B7	B	A	B	A
		492.8	A	B	B	A	B7	B	A	B	A
		492.9	B1	B	B	A	B8	B	A	B	A
		492.10	A	B	B	A	B7	B	A	B	A
		780.1	B1	B	B	A	B5	B	B	B	A
		780.2	B1	B	B	A	B4	B	B	B	A
		780.3	B3	B	B	A	B6	B	B	B	A
		780.4	B2	B	B	A	B6	B	B	B	A
B	**Northern**	**780.5**	B2	B	B	A	B6	B	B	B	A
	Norway	**780.6**	B2	B	B	A	B7	B	B	B	A
		780.7	B1	B	B	A	B5	B	A	B	A
		780.8	B1	B	B	A	B9	B	B	B	A
		780.9	B1	B	B	A	B9	B	B	B	A
		780.10	B3	B	B	A	B8	B	B	B	A
		800.1	A	B	A	A	B5	A	B	B	A
		800.2	A	B	B	A	B6	A	B	A	A
		800.3	A	B	B	A	B5	A	B	A	A
		800.4	A	B	B	A	B6	A	A	A	A
A	**Sweden**	**800.5**	A	B	B	A	B6	A	A	A	A
		800.6	nr	nr	B	nr	B6	A	B	A	A
		800.7	nr	nr	B	nr	B7	A	B	A	A
		800.8	nr	nr	B	nr	B6	A	B	A	A
		800.9	nr	nr	B	nr	B6	A	B	A	A
		800.10	nr	nr	B	nr	B6	A	B	A	A
		782.1	A	B	B	A	B6	A	B	B	A
		782.2	A	B	B	A	B6	A	B	B	A
		782.3	A	B	B	A	B6	A	B	A	A
		782.4	A	B	B	A	B6	A	B	B	A
A	**Sweden**	**782.5**	A	B	B	A	B6	A	B	A	A
		782.6	A	B	B	A	B6	A	B	A	A
		782.7	A	B	B	A	B5	A	B	A	A
		782.8	A	B	B	A	B6	A	B	B	A
		782.9	A	B	B	A	B7	A	B	B	A
		782.10	A	B	B	A	B5	A	B	A	A
		790.1	A	B	B	A	B4	A	B	B	A
		790.2	A	B	B	A	B4	A	B	B	A
		790.3	A	B	C	A	B4	A	B	B	A
		790.4	A	B	C	A	B5	A	B	B	A
A	**Sweden**	**790.5**	A	B	C	A	B4	A	B	B	A
		790.6	A	B	C	A	B4	A	A	B	A
		790.7	A	B	C	A	B4	A	A	B	A
		790.8	A	B	C	A	B5	A	B	B	A
		790.9	A	B	C	A	B4	A	B	B	A
		790.10	A	B	C	A	B4	A	B	B	A
		779.1	A	B	A	A	B9	A	B	B	A
		779.2	A	B	A	A	B8	A	B	B	A
		779.3	A	B	B	A	B5	A	A	B	A

		779.4	A	B	B	A	B5	A	A	B	A
A	**Sweden**	**779.5**	A	B	B	A	B5	A	B	B	A
		779.6	A	B	B	A	B5	A	B	B	A
		779.7	A	B	B	A	B5	A	A	B	A
		779.8	A	B	B	A	B7	A	A	A	A
		779.9	A	B	B	A	B8	A	A	B	A
		779.10	A	B	B	A	B7	A	B	B	A
		797.1	A	B	B	A	B6	A	A	B	A
		797.2	A	B	B	A	B7	A	A	B	A
		797.3	A	B	B	A	B5	B	B	B	A
		797.4	A	B	B	A	B6	B	B	B	A
A	**Sweden**	**797.5**	A	B	B	A	B5	B	B	B	A
		797.6	A	B	B	A	B6	B	B	B	A
		797.7	A	B	B	A	B7	A	A	B	A
		797.8	A	B	B	A	B7	A	B	B	B
		797.9	A	B	B	A	B6	B	B	B	A
		797.10	A	B	B	A	B6	A	A	B	A
		801.1	A	B	B	A	B4	A	A	B	A
		801.2	A	B	B	A	B7	A	A	B	A
		801.3	A	B	B	A	B9	A	B	B	B
		801.4	A	B	B	A	B6	A	A	B	A
A	**Sweden**	**801.5**	A	B	B	A	B5	A	A	B	A
		801.6	A	B	B	A	B4	A	A	B	A
		801.7	A	B	B	A	B7	A	A	B	A
		801.8	A	B	B	A	B10	A	B	B	B
		801.9	A	B	B	A	B10	A	B	B	B
		801.10	A	B	B	A	B7	B	B	B	A
		781.1	A	B	B	A	B6	A	B	B	A
		781.2	A	B	B	A	B5	A	A	B	A
		781.3	A	B	B	A	B3	A	A	B	A
		781.4	nr	nr	B	nr	B5	A	A	B	A
A	**Sweden**	**781.5**	nr	nr	B	nr	B3	A	A	B	A
		781.6	nr	nr	B	nr	B4	A	A	B	A
		781.7	nr	nr	B	nr	B3	A	A	B	A
		781.8	nr	nr	B	nr	B4	A	A	B	A
		781.9	nr	nr	B	nr	B3	A	A	B	A
		781.10	nr	nr	nr	nr	nr	nr	nr	nr	nr
		802.1	A	B	B	A	B7	B	B	A	A
		802.2	A	B	B	A	B7	B	B	A	B
		802.3	A	B	B	A	B10	B	B	A	B
		802.4	A	B	B	A	B7	A	B	A	A
A	**Sweden**	**802.5**	A	B	B	A	B6	A	B	A	A
		802.6	A	B	B	A	B7	B	B	A	B
		802.7	A	B	B	A	B6	B	B	A	B
		802.8	A	B	B	A	B6	B	B	A	A
		802.9	A	B	B	A	B7	B	B	A	B
		802.10	A	B	B	A	B6	B	B	A	B
		798.1	A	B	B	A	B6	A	B	B	A
		798.2	A	B	C	A	B4	A	B	B	A
		798.3	A	B	C	A	B5	A	B	B	A
		798.4	A	C	C	A	B5	A	B	B	A
A	**Sweden**	**798.5**	A	B	C	A	B4	A	B	B	A

		798.6	A	B	B	A	B7	A	B	B	A
		798.7	A	B	C	A	B5	A	B	B	A
		798.8	A	B	C	A	B5	A	B	B	A
		798.9	A	B	B	A	B5	A	B	B	A
		798.10	A	B	C	A	B5	A	B	B	A
		805.1	B4	B	C	B1	B5	A	B	B	A
		805.2	B1	B	A	A	B6	A	B	B	A
		805.3	B2	B	B	B2	B6	A	B	B	A
		805.4	B4	B	B	B1	B5	A	B	B	A
B	**Sweden**	**805.5**	B2	B	B	B1	B5	A	B	B	A
		805.6	A	B	B	A	B6	A	B	B	B
		805.7	B1	B	B	B1	B6	A	B	B	A
		805.8	B3	B	B	A	B5	A	B	B	B
		805.9	B4	B	C	B1	B5	A	B	B	B
		805.10	B3	B	B	A	B5	A	B	B	B
		649.1	A	B	B	A	B6	B3	B	B	A
		649.2	B2	B	B	A	B8	B2	B	B	A
		649.3	B3	B	B	A	B7	B2	B	B	A
		649.4	B3	B	B	A	B6	B4	B	B	A
B	**France**	**649.5**	B3	B	B	A	B6	B4	B	B	A
		649.6	B3	B	B	A	B6	B2	B	B	A
		649.7	B3	B	B	A	B6	B2	B	B	A
		649.8	B3	B	B	A	B6	B3	B	B	A
		649.9	B4	B	B	A	B7	B3	B	B	A
		649.10	B2	B	B	A	B7	B3	B	B	A
		842.1	A	B	B	A	B8	B2	B	B	A
		842.2	A	B	B	A	B10	B4	B	B	A
		842.3	A	B	B	A	B7	B2	B	B	A
		842.4	A	B	B	A	B7	B2	B	B	A
B	**St Pierre &**	**842.5**	A	B	B	A	B5	B2	B	B	A
	Miquelon	**842.6**	A	B	C	A	B7	B2	B	B	A
		842.7	A	B	B	A	B5	B3	B	B	A
		842.8	A	B	B	A	B7	B4	B	B	A
		842.9	A	B	B	A	B7	B4	B	B	A
		842.10	A	B	B	A	B6	B3	B	B	A
		642.1	B3	B	B	B1	B4	B2	B	B	A
		642.2	B3	B	B	A	B4	B2	B	B	A
		642.3	B5	B	B	A	B3	B2	B	B	A
		642.4	B4	B	B	B3	B5	B1	B	B	A
B	**France**	**642.5**	B3	B	B	B1	B5	B1	B	B	A
		642.6	nr	B	B	nr	B6	B2	B	B	A
		642.7	nr	B	nr	nr	nr	nr	B	B	A
		642.8	nr	B	nr	nr	nr	nr	B	B	A
		642.9	nr	B	nr	nr	nr	nr	A	B	A
		642.10	nr	B	nr	nr	nr	nr	B	B	A
		534.1	B1	B	B	A	B9	B4	B	B	A
		534.2	A	B	B	A	B9	B6	B	B	A
		534.3	B4	B	B	A	B10	B7	B	B	A
		534.4	B2	B	B	A	B10	B4	B	B	A
B	**Denmark**	**534.5**	A	B	B	A	B8	B6	B	B	A
		534.6	A	B	B	A	B8	B6	A	B	A
		534.7	A	B	B	A	B9	B6	A	B	A

		534.8	A	B	B	A	B12	B7	A	B	A
		534.9	B3	B	B	A	B8	B5	A	B	A
		534.10	A	B	B	A	B10	B6	A	B	A
		541.1	B2	B	B	B2	B6	B6	B	B	A
		541.2	B5	B	B	A	B6	B4	B	B	A
		541.3	B4	B	B	A	B7	B5	B	B	A
		541.4	B7	B	B	A	B6	B3	B	B	A
B	**Far East Russia**	**541.5**	B5	B	B	B1	B6	B6	B	B	A
		541.6	nr	nr	B	nr	B6	B3	B	B	A
		541.7	nr	nr	B	nr	B6	B6	B	B	A
		541.8	nr	nr	B	nr	B6	B6	B	B	A
		541.9	nr	nr	B	nr	B6	B6	B	B	A
		541.10	nr	nr	B	nr	B7	B5	B	B	A
		560.1	B8	B	B	B1	B8	B1	B	B	A
		560.2	B8	B	B	A	B7	B2	B	B	A
		560.3	B12	B	B	A	B7	B2	B	B	A
		560.4	B4	B	B	A	B7	B4	B	B	A
B	**Far East Russia**	**560.5**	B7	B	B	A	B5	B4	B	B	A
		560.6	B8	B	B	A	B8	B1	B	B	A
		560.7	B9	B	B	A	B6	B4	A	B	A
		560.8	B14	B	B	B1	B6	B9	B	B	A
		560.9	B14	B	B	B1	B6	B4	B	B	A
		560.10	B10	B	B	A	B8	B1	B	B	A
		654.1	B9	B	B	A	B8	B2	B	B	A
		654.2	B4	B	B	A	B7	B5	B	B	A
		654.3	B4	B	B	A	B7	B5	A	B	A
		654.4	B2	B	B	A	B5	B2	A	B	A
B	**France**	**654.5**	B3	B	B	A	B10	B5	B	B	A
		654.6	B5	B	B	A	B8	B5	B	B	A
		654.7	B3	B	B	A	B9	B5	B	B	A
		654.8	B5	B	B	A	B9	B3	B	B	A
		654.9	B4	B	B	A	B5	B3	B	B	A
		654.10	B4	B	B	A	B9	B4	B	B	A
		663.1	B5	B	B	A	B7	B6	B	B	A
		663.2	B6	B	B	A	B6	B4	B	B	A
		663.3	A	B	B	A	B7	B4	B	B	A
		663.4	B1	B	B	A	B8	B4	B	B	A
B	**France**	**663.5**	B4	B	B	A	B6	B3	B	B	A
		663.6	B4	B	B	A	B7	B4	B	B	A
		663.7	B4	B	B	A	B7	B3	B	B	A
		663.8	B2	B	B	A	B8	B3	B	B	A
		663.9	B3	B	B	A	B7	B3	B	B	A
		663.10	B5	B	B	A	B6	B4	B	B	A

Appendix III

		Character number	9	10	11	12
Molecular group	**Country**	**Specimen number**	**Upper dorsal costa roughness**	**Upper dorsal costa roughness**	**Leaf curvature**	**Leaf lenght**
A	**Sweden**	**617.1**	B2	A	A	54,00
		617.2	B2	A	A	49,2
		617.3	A	nr	A	45,6
		617.4	C	A	A	57,6
		617.5	C	A	A	48
B	**France**	**644.1**	B2	A	B	84
		644.2	B2	A	B	90
		644.3	B2	A	B	110,4
		644.4	B2	A	B	100,8
		644.5	B2	A	B	86,4
B	**Japan**	**816.1**	B5	A	B	132
		816.2	B2	A	B	144
		816.3	B2	A	B	105,6
		816.4	B4	A	B	124,8
		816.5	B2	A	B	103,2
A	**Western Russia**	**559.1**	B2	A	B	100,8
		559.2	B4	A	B	96
		559.3	B2	A	B	103,2
		559.4	B2	A	B	98,4
		559.5	B2	A	B	96
A	**Canada**	**492.1**	B2	A	B	84
		492.2	B2	A	B	96
		492.3	B2	A	B	84
		492.4	B2	A	B	81,6
		492.5	B2	A	B	86,4
B	**Northern Norway**	**780.1**	A	nr	A	43,2
		780.2	B2	A	A	64,8
		780.3	B2	A	A	52,8
		780.4	B2	A	A	81,6
		780.5	B2	A	A	67,2
A	**Sweden**	**800.1**	A	nr	A	73,2
		800.2	A	nr	A	72
		800.3	A	nr	A	68,4
		800.4	A	nr	A	72
		800.5	A	nr	A	75,6
A	**Sweden**	**782.1**	C	A	B	86,4
		782.2	C	A	B	86,4
		782.3	C	A	B	91,2
		782.4	C	A	B	86,4
		782.5	nr	nr	B	60
A	**Sweden**	**790.1**	C	A	A	60
		790.2	C	A	A	52,8

		790.3	C	A	A	62,4
		790.4	C	A	A	60
		790.5	C	A	A	51,6
A	**Sweden**	**779.1**	C	A	A	72
		779.2	A	nr	A	69,6
		779.3	A	nr	A	78
		779.4	B2	A	A	69,6
		779.5	C	A	A	72
A	**Sweden**	**797.1**	C	A	A	62,4
		797.2	A	nr	A	57,6
		797.3	B2	A	A	72
		797.4	nr	nr	A	67,2
		797.5	C	A	A	67,2
A	**Sweden**	**801.1**	C	A	A	73,2
		801.2	C	A	A	75,6
		801.3	B2	A	A	72
		801.4	C	A	A	72
		801.5	B2	A	A	73,2
A	**Sweden**	**781.1**	A	nr	A	39,6
		781.2	A	nr	A	48
		781.3	A	nr	A	40,8
		781.4	nr	nr	nr	0
		781.5	nr	nr	nr	0
A	**Sweden**	**802.1**	A	nr	A	55,2
		802.2	A	nr	A	52,8
		802.3	A	nr	A	56,4
		802.4	A	nr	A	51,6
		802.5	A	nr	A	52,8
A	**Sweden**	**798.1**	B2	A	A	55,2
		798.2	B2	A	A	72
		798.3	B2	A	A	64,8
		798.4	B2	A	A	66
		798.5	B2	A	A	48
B	**Sweden**	**805.1**	B2	A	B	67,2
		805.2	B2	A	B	67,2
		805.3	B2	A	B	70,8
		805.4	B2	A	B	66,72
		805.5	B2	A	B	70,32
B	**France**	**649.1**	C	A	B	98,4
		649.2	C	A	B	108,24
		649.3	C	A	B	60,24
		649.4	C	A	B	0
		649.5	C	A	B	0
B	**St Pierre & Miquelon**	**842.1**	C	A	B	100,8
		842.2	C	A	B	99,6
		842.3	C	A	B	105,6
		842.4	C	A	B	94,8
		842.5	C	A	B	106,8
B	**France**	**642.1**	B2	A	B	76,8
		642.2	B2	A	B	87,36
		642.3	B2	A	B	79,2
		642.4	B2	A	B	72

		642.5	nr	nr	nr	0
B	**Denmark**	**534.1**	C	A	B	132
		534.2	C	A	B	127,2
		534.3	C	A	B	121,2
		534.4	C	A	B	124,8
		534.5	C	A	B	110,4
B	**Far East Russia**	**541.1**	C	A	B	103,2
		541.2	C	A	B	105,6
		541.3	C	A	B	88,8
		541.4	C	A	B	98,4
		541.5	C	A	B	116,4
B	**Far East Russia**	**560.1**	C	A	B	88,8
		560.2	C	A	B	96
		560.3	C	A	B	0
		560.4	C	A	B	96
		560.5	C	A	B	97,2
B	**France**	**654.1**	C	A	B	106,8
		654.2	C	A	B	96
		654.3	C	A	B	112,8
		654.4	C	A	B	105,6
		654.5	C	A	B	91,2
B	**France**	**663.1**	C	A	B	100,8
		663.2	C	A	B	110,4
		663.3	C	A	B	93,6
		663.4	C	A	B	105,6
		663.5	C	A	B	97,2

Chapitre III : Considérations évolutives et taxinomiques sur quelques espèces

Le genre *Dicranum,* riche de 132 espèces, possède des caractères évolutifs originaux : organes dédiés à la reproduction végétative ou bien le nanisme des plantes mâles. Ainsi, environ 25 % des espèces du genre possèdent des mâles nains. Cependant, aucune information sur la biologie des espèces n'est disponible pour près de 50 % des espèces. De plus, un quart de ces espèces n'est connu qu'à travers la description princeps du taxon. On peut dresser ainsi l'état des lieux suivant :

(1) pour les taxons les plus communs, l'écologie, la biologie et le cycle de vie des espèces sont bien documentés,

(2) pour les espèces peu communes, la diagnose des espèces a été révisée, localement, à la lumière des nouvelles collectes,

(3) pour la moitié des taxons du groupe, dont les descriptions datent majoritairement de la fin du 18ème siècle, aucune information n'est disponible et aucune nouvelle observation morphologique n'a été faite à la suite de la publication de la diagnose princeps de l'espèce.

Le travail de recherche effectué au cours de ces trois ans de thèse a nécessité l'examen d'un certain nombre de spécimens types et leurs comparaisons, base à une révision taxonomique du groupe *Dicranum*. De ces observations, des incohérences entre la morphologie et les données de la littérature ont été mises en évidence. Ce chapitre va donc s'intéresser à décrire, préciser et argumenter les caractères reproductifs et morphologiques de quelques espèces.

Article 4: Pichonet, A. & S. Robbert Gradstein, Male dwarfism in the genus *Dicranum* (Dicranaceae) – a review

Cryptogamie, Bryologie - accepted

Male dwarfism in the genus *Dicranum* (Dicranaceae) – a review

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Abstract – Understanding of male dwarfism in mosses is reviewed with special reference to the genus *Dicranum*. Dwarf males occur in about 20% of *Dicranum* species. Most species seem to be obligately nannadrous; in two species (*D. bonjeanii*, *D. scoparium*) male plant size may be normal or dwarfed. Variation in male plant size in *Dicranum* seems to be environmentally controlled. Male dwarfism in mosses is induced by genetic or environmental factors although the mechanisms leading to male dwarfism are poorly understood. Evidence suggests that male dwarfism increases reproductive success in dioicous species but many questions remain unanswered.

Bryophyta / dioicy / dwarf male / male dwarfism / nannandry / reproductive strategy / sexual dimorphism / *Dicranum*

INTRODUCTION

The occurrence of sexual reproduction in bryophytes was first suggested by Johann Jacob Dillenius (1684-1747) in his *Catalogus Plantarum circa Gissam sponte nascentium* (1719). His observations came only 25 years after the discovery of sexual reproduction in plants (Camerarius, 1694). Dillenius recognized two structures for sexual propagation in the moss now called *Aulacomnium androgynum* (Hedw.) Schwägr., the gemma stalk of this species being considered the female organ and the sporophyte the male organ. Ten years later, in his *Nova Plantarum Genera* (1729), Pier Antonio Micheli (1679-1737) published first observations on sex organs in liverworts and hornworts. In accordance with the opinion of Dillenius, Micheli interpreted the sporophyte as the male organ and various asexual reproductive structures, such as gemma cups in *Marchantia* and leaf-born gemmae in *Scapania*, *Radula* and *Calypogeia*, as female organs (Margadant, 1973). Micheli also illustrated archegonia, antheridia and paraphyses but did not name these structures nor

describe their function. The function of the sporangium as the spore-producing of the bryophytes was first recognized in 1747 by Casimir Christoph Schmidel (1718-1792). Schmidel also correctly interpreted the antheridium as the male organ but did not observe the sperm cells (Schofield, 1985). Subsequently, Johann Hedwig (1730-1799) proposed the female function of the archegonia from his observations on growth and structure of the sporophyte and the spores of mosses in his *Fundamentum Historiae Naturalis Muscorum Frondosorum* (1782). Hedwig also assumed that antheridia produce sperm (Margadant, 1973) although proof for this notion did not come until much later, in 1834, when Franz Joseph Andreas Nicolaus Unger (1800-1870) observed the release of antherozoids from antheridia in the genus *Sphagnum*. The sexuality and life cycle of the bryophytes was finally resolved by Wilhelm Hofmeister (1824-1877) in his epoch-making *Vergleichende Untersuchungen* (1851) in which he described the egg cell within the archegonium and the two distinct phases of the life cycle, comparing these with the life cycle of ferns and gymnosperms.

Although mosses, liverworts and hornworts share a similar life cycle and sexual reproduction by means of antheridia and archegonia, different reproductive strategies are observed among the three groups. An unusual reproductive feature distinguishing mosses from liverworts and hornworts is the occurrence in mosses of sexual dimorphism by male dwarfism or *nannandry* (derived from Greek *nann* = dwarf, *andro* = male), *i.e.* the growth of dwarfed male plants on the stems or leaves of normal-sized female plants. Size of the dwarf males varies from less than half to equal the length of the leaves of the female plant. Some dwarf males have very few leaves and contain a single antheridium while others have many of both (Ramsay, 1979).

Male dwarfism in mosses was first detected by Wilhelm Philipp Schimper (1808-1880), who in his *Bryologia Europeae* described the occurrence of annual male plants originating from the “adventive roots” of perennial female plants or from spores in the genus *Dicranum* (Schimper, 1837; see also Schimper, 1850). Schimper observed that the dwarf male plants occurred in the autumn and in the spring on fertile female plants but never on normal-sized male plants. He also noted that in some species of *Dicranum* normal-sized male plants are lacking and replaced by male “buds” originating from the the tomentum of the female plants. Forty-seven years later, Henri Philibert (1822-1901) described dwarf males in the mosses *Fissidens decipiens* De Not. (= *F. dubius* P. Beauv.) and *Camptothecium lutescens* (Gedw.) Schimp. (Philibert, 1883). Both Schimper and Philibert speculated that the dwarf male plants originated from female protonema, and Philibert (1883) in addition suggested that they might have originated from the female gametophore. Recent studies, however, have shown that dwarf males originate from spores (e.g., Ramsay, 1979; Une, 1985a,b).

Hedenäs & Bisang (2011) in a study on male dwarfism in pleurocarpous mosses found that nannandry was much more common than hitherto reported. It has been recorded from twenty-two moss families and may be a useful taxonomic feature at the family level. Male dwarfism occurs in all species of the genus *Garovaglia* (During, 1977) but in the majority of genera (and families) with male dwarfs the phenomenon is expressed in only part of the species. Hedenäs & Bisang (2011) also found that the majority of pleurocarpous species with dwarf males were facultatively nannandrous and produced normal-sized male plants as well. Species with obligate dwarf males appeared to be rare.

In the acrocarpous mosses male dwarfism has been reported in Dicranaceae, Fissidentaceae, Leucobryaceae and Orthotrichaceae. Much work has been done on male dwarfism in the orthotrichaceous genus *Macromitrium* where male dwarfism is associated with anisospory (e.g., Ernst-Schwarzenbach, 1939; Ramsay, 1979; Une, 1985a,b,c). In this paper we explore the occurrence of male dwarfism in the genus *Dicranum* (Dicranaceae). In addition, the biology of male dwarfism is briefly discussed.

MALE DWARFISM IN *DICRANUM*

Male dwarfism in *Dicranum* was first reported by Schimper (1837) who described the occurrence of dwarf males in five species (Table 1): *D. majus, D. robustum, D. schraderi* (now = *D. undulatum* Turn.), *D. spurium* and *D. undulatum*. He observed that normal-sized male plants occur as well in *D. majus D. robustum* and *D. undulatum*, but never in *D. schraderi* and *D. spurium* where they were always replaced by the small, bud-like male plants growing on the tomentous stems of the female plants ("*gemmulae masculae in tomento nascentes plus minus copiosae*").

Within 131 currently accepted species in *Dicranum* (van der Wijk *et al.*, 1962, 1969; Tropicos, 2010), 25 species are known to produce dwarf males, 21 lack male dwarfism and in more than 55 species male plants are unknown (Table 1). The occurrence of dwarf males in one species of *Dicranum*, *D. fragilifolium*, remains controversial. Bellolio-Trucco & Ireland (1990) reported the occurrence of normal-sized male plants in this species while Gao *et al.* (1999) recorded dwarf males. Only two species of *Dicranum* (*D. scoparium*, *D. bonjeanii*) are known to produce both normal-sized and dwarf male plants (Loveland, 1956; Briggs, 1965; Table 1). The apparent rarity of facultative nannandry in *Dicranum* is in contrast with pleurocarpous mosses where it is common (Hedenäs & Bisang, 2011).

Dwarf males have been recorded in ten genera of the Dicranaceae (Ramsay, 1979; Gao *et al.*, 1999), including four that are closely allied to *Dicranum*: *Dicranoloma,*

Eucamptodontopsis, *Holomitrium* and *Pseudochorisodontium* (LaFarge *et al.*, 2002). Male dwarfism is apparently lacking in the dicranaceous genera *Chorisodontium*, *Orthodicranum* and *Paraleucobryum*.

The expression of male dwarfism in *Dicranum* was first studied by Loveland (1956). He found that in the facultatively nannandrous *D. scoparium* male plant size varies according to the proximity of the female shoot. Loveland concluded that at least two different mechanisms may lead to male dwarfism in this genus: 1) genetic or chromosomal determinism, and 2) chemical influences. Briggs (1964) confirmed that development of dwarf males in *D. scoparium* is environmentally controlled and also observed this phenomenon in *D. bonjeanii*. The influence of the environmental factors on the expression of male dwarfism has also been shown in *Macromitrium* (see below).

Since the pioneering work of Loveland and Briggs, no further experiments have been conducted on the mechanism of male dwarfism in *Dicranum*. However, several studies were carried out on the reproductive biology and phenology of nannandrous *Dicranum* species (Briggs, 1965; Hughes, 1980; Sagmo Solli *et al.*, 1998, 2000; Ehrlen *et al.*, 2000; Bisang & Ehrlen, 2002). Hughes (1980) showed that in the polysetous *D. majus* and *D. scoparium* dwarf males never occurred in association with female plants without sporophytes. In a study on the reproductive phenology of *D. majus*, Sagmo Solli *et al.* (1998, 2000) confirmed that dwarf males are annuals. Moreover, these authors demonstrated that the occurrence of dwarf males increases the number of plants with sporophytes and that the proportion of fertilized archegonia increases with the number of dwarf males on female shoots.

GENERAL DISCUSSION

Male dwarfism may be induced by genetic factors, (Ernst-Schwarzenbach, 1939; Une, 1985a) environmental factors (Une, 1985a) and unfavourable nutrient conditions (Woesler, 1935). It is considered a strategy to enhance reproduction in dioicous species, which include about 40% of all moss species (Wyatt, 1994). Suppression of sexuality is often observed in these dioicous taxa due to the spatial separation of male and female plants and their limited fertilization range (Longton & Schuster, 1983, Bisang *et al.*, 2004). Male dwarfism may counterbalance the reproductive constraints in these taxa, however may also lead to an increase in the rate of inbreeding. Following fertilization of the haploid parental plants by offspring of the F_1-generation, recessive alleles may become expressed in the offspring of the F_2-generation, resulting in large phenotypic variability (Mogensen, 1981; Hedenäs & Bisang, 2011). The latter authors suggested that the taxonomic difficulties encountered in genera with

male dwarfism (e.g. *Dicranum*, *Macromitrium*) may be due to the commonness of back-crossing in these groups and the resulting phenotypic complexity.

Evidence for increase in fertilization and outbreeding when males are closer to the female plants has been demonstrated in *Macromitrium* (Une, 1985b). The latter author also found a correlation between temperature and male plant size in Japanese *M. gymnostomum* Sull. *et* Lesq. and *M. japonicum* Dozy *et* Molk. In these two species, normal-sized male plants are only found in regions where mean January temperature is higher than 6 °C. This suggests that male dwarfism in these species is an adaptation to low temperatures. Une (1985c) also found that germination of male spores at low temperatures is slower than that of female spores.

Male dwarfism occurs in both isosporous and anisosporous mosses. Anisospory is always linked with sexual dimorphism but the opposite is not true (Ramsay, 1979; Mogensen, 1981). In anisosporous *Macromitrium* species cultivated in vitro, dwarf males were always obtained from microspores, whether grown isolated or together with female plants (Une, 1985a). In isosporous *Macromitrium*, Une (1985a) showed that the initiation of male dwarfism was induced by a phytohormone from auxin family (2, 4-dichlorophenoxyacetique acid: 2, 4D) known to increase the rate of DNA, RNA and protein synthesis. He concluded that the development of dwarf males in the isosporous species was hormonally regulated. He also found that small spores of isosporous *Macromitrium* give rise to dwarf males much more often that randomly sampled spores under phytohormone regulation (75% of small spores *vs* 25% of randomly sampled spores). Une (1985a) concluded that small spores of isosporous *Macromitrium* are male. Based on his results, Une (1985a) proposed a new terminology for male dwarfism, taking into account underlying developmental mechanisms. Since Schimper (1837), various terms have been employed to characterize species with dwarf males (e.g. "pseudo-monoicous", "pseudo-autoicous", "phyllodioïcous"). Following Une, genetically determined dwarf males are called “eunanandrous” and those influenced by female phytohormones “pseudonanandrous”.

Dwarf males seem to generally establish on female plants of the same species, and a single female plant may host a variety of dwarf males, including siblings or conspecific populations. Pedersen *et al.* (2006) in a molecular study using ITS markers found that the dwarf males of *Garovaglia elegans* (Dozy *et* Molk.) Bosch *et* Sande Lac. and *G. powellii* Mitt. were conspecific with their respective female hosts. The results needed confirmation by further sampling, however, as phylogenetic relationships within the clade containing the two species were poorly resolved. Growth of dwarf males on either the stem or the leaves of the female plant seems to depend on the suitability of leaves and stems for the establishment and

germination of the spores (Ernst-Schwarzenbach, 1939). In a study on the occurrence of male dwarfism in the Ptychomniales, Pedersen & Newton (2007) found that male dwarfism was more common in plants with rugose or toothed dorsal leaf surfaces than in plants with smooth leaves. They proposed that the roughened leaf surfaces promoted the establishment of spores, and thus male dwarfism. The mechanisms inhibiting the germination of "foreign" spores and/or stimulating the germination of those of the same species remain unknown, however.

CONCLUDING REMARKS

Male dwarfism is known in about 20% of *Dicranum* species but in almost half of the species of the genus male plants remain unknown. Phenotypic plasticity of male plant size has been observed in two species of *Dicranum* (*D. bonjeanii*, *D. scoparium*), all other species of the genus with dwarf males seem to be obligately nannandrous. The percentage of obligately nannandrous species in *Dicranum* is higher than in pleurocarpous mosses where it is rare (Hedenäs & Bisang, 2011). The mechanisms leading to male dwarfism are still poorly understood and may include genetic, environmental and physiological factors. In their recent overview of nannandry in pleurocarpous mosses, Hedenäs & Bisang (2011) emphasized that male dwarfism in bryophytes is an overlooked phenomenon. In the acrocarpous mosses, where male dwarfism occurs in four different families, nannandry has been little studied with exception of the genus *Macromitrium*. A comprehensive study on male dwarfism in acrocarpous mosses would be desirable and might contribute to a better understanding of the origin, evolution and biological relevance of this neglected phenomenon in mosses.

Acknowledgements. Thanks are due to Denis Lamy for this help and critical comments on the manuscript, and to two anonymous reviewers for numerous valuable suggestions leading to considerable improvement of the paper. We also thank Amandine Allard for assistance in checking and digitalizing the *Dicranum* types in herbarium PC. This paper is part of the first author's doctoral dissertation.

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(captions)

Table 1. List of accepted species in *Dicranum* (van der Wijk *et al*. 1962, 1969; Tropicos, 2010) and male plant status. DWF = dwarf males; N = normal-sized males; U = male plant status unknown; * = species known only from the original diagnosis.

Figs 1-**3. 1**: Female plant of *Dicranum undulatum* Schrad. *ex* Brid. **2**: Dwarf males of *Dicranoloma brevisetum* (Dozy *et* Molk.) Paris (after Fleischer, 1900-1922). **3**: Dwarf males on a female stem in *Dicranum undulatum* Schrad. *ex* Brid. (scale: 0.5 cm).

Figs 4-7**. 4**: Dwarf male of *Dicranum undulatum* Schrad. *ex* Brid. (scale: 3 mm). **5**: Paraphyses and antheridia of the dwarf male of *D. undulatum* (scale: 300 µm). **6**: Antheridia of the dwarf male of *D. undulatum* (scale: 150 µm). **7**: Dwarf male of *D. lorifolium* Mitt. (isotype: PC0128679) (scale: 250 µm).

Fig. 8. Determinisms driving male dwarfism, modified from Une (1985a)

Table 1

	Species	**Male plant status**	**Reference**
1	*Dicranum acanthoneurum* Müll. Hal.	U	*Flora* 73: 474. 1890
2	*Dicranum acutifolium* (Lindb. *et* Arnell) C.E.O. Jensen	DWF	*Herzogia* 17: 179-197. 2004
3	**Dicranum alpinum* (P. Beauv.) Brid.	U	*Muscologia Recentiorum Supplementum* 1: 208. 1806
4	*Dicranum arcuatipes* Müll. Hal.	U	*Genera Muscorum Frondosorum* 299. 1900
5	*Dicranum assamicum* Dixon	N	*Moss Flora of China*. 1: 165. 1999
6	*Dicranum atratum* Geh.	U	*Flora* 62: 473. 1879
7	**Dicranum atro-viride* Cardot	U	*Annales botanici societatis zoologicae-botanicae Fennicae "Vanamo"* 9: 41. 1937
8	**Dicranum beyrichianum* (Duby) Hampe	U	*Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn* 9-10: 255. 1878
9	*Dicranum bonjeanii* De Not.	DWF, N	*Herzogia* 17: 179-197. 2004.
10	*Dicranum borbonicum* Renault *et* Cardot	U	*Prodrome de la Flore Bryologique de Madagascar des Mascareignes et des Comores* 160. 1898
11	*Dicranum braunsiae* Müll. Hal.	U	*Genera Muscorum Frondosorum* 290. 1900
12	*Dicranum brevifolium* (Lindb.) Lindb.	DWF	*Herzogia* 17: 179-197. 2004
13	*Dicranum caesium* Mitt.	U	*Transactions of the Linnean society of London, Botany* 3: 156. 1891
14	**Dicranum caldense* Müll. Hal.	U	*Hedwigia* 39: 250. 1900
15	**Dicranum capillatus* (Hook. *et* Wilson) L.C. Beck	U	*Transactions and proceedings of the New Zealand institute* 25: 301. 1893
16	**Dicranum carneum* Blandow	U	*Deutschlands Flora*, Abtheilung II, Cryptogamie 10: ic. 1809
17	*Dicranum cheoi* E.B. Bartram	DWF	*Moss Flora of China*. 1: 167. 1999
18	**Dicranum clericii* Brizi	U	*Bollettino de società geologica Italiana* 9: 365. 1892
19	**Dicranum columbiae* (Kindb.) Renauld *et* Cardot	U	*Revue bryologique* 19: 77. 1892
20	*Dicranum condensatum* Hedw.	DWF	*Herzogia* 17: 179-197. 2004
21	**Dicranum conglomeratum* (Brid.) Wallr.	U	*Flora Cryptogamica Germaniae* 1:

			169. 1831
22	**Dicranum craigieburnense* R. Br. bis	U	*Transactions and proceedings of the New Zealand institute* 29: 457. 1897
23	*Dicranum crassifolium* Sérgio, Ochyra *et* Seneca	DWF	*Herzogia* 17: 179-197. 2004
24	*Dicranum crispifolium* Müll. Hal.	N	*Botanische Zeitung (Berlin)* 22: 349. 1864
25	*Dicranum crispatulum* (Roll) Kindb.	U	*European and N. American Bryineae (Mosses*) 2: 189. 1897
26	*Dicranum decumbens* Thwaites *et* Mitt.	U	*Journal of the Linnean society, Botany* 13: 296. 1873
27	*Dicranum deflexicaulon* Müll. Hal.	U	*Linnaea* 38: 589. 1874
28	*Dicranum delavayi* Besch.	U	*Revue bryologique* 18: 88. 1891
29	*Dicranum dilatinerve* Cardot *et* P. de la Varde	U	*Revue bryologique* 49: 35. 1922
30	*Dicranum diplospiniferum* C. Gao *et* C. W. Aur	U	*Bulletin of botanical laboratory of North-Eastern forestry institute* 7: 99. 1980.
31	*Dicranum dispersum* Engelmark	DWF	*Herzogia* 17: 179-197. 2004
32	*Dicranum drummondii* Müll. Hal.	DWF	*Herzogia* 17: 179-197. 2004
33	*Dicranum dubium* Thér. *et* Dixon	U	*Revue bryologiqu*e 48: 12. 1921
34	*Dicranum eggersii* Müll. Hal.	U	*Genera Muscorum Frondosorum* 287. 1900
35	*Dicranum elongatum* Schleich. *ex* Schwägr.	N	*Herzogia* 17: 179-197. 2004
36	*Dicranum filum* Bory	U	*Voyage dans les quatre principales Îles des Mers d'Afrique* 3: 17. 1804
37	*Dicranum flagellare* Hedw.	N	*Herzogia* 17: 179-197. 2004
38	*Dicranum fragilifolium* Lindb.	DWF, N	*Herzogia* 17: 179-197. 2004
39	*Dicranum fragillimum* Warnst.	U	*Hedwigia* 57: 78. 14. 1915
40	*Dicranum frigidum* Müll. Hal.	DWF	Moss Flora of Central America. Tropicos®
41	*Dicranum fulvum* Hook.	N	*Herzogia* 17: 179-197. 2004
42	*Dicranum fuscescens* Turner	N	*Herzogia* 17: 179-197. 2004
43	*Dicranum gonoi* Cardot	U	*Bulletin de la Société Botanique de Genève*, Sér. 2 1: 121. 1909
44	*Dicranum gregoryi* B. H. Allen	U	*The bryologist* 91: 91. 1988
45	*Dicranum groenlandicum* Brid.	N	*Herzogia* 17: 179-197. 2004
46	*Dicranum hamulosum* Mitt.	N	*Moss Flora of China*.1: 173-174. 1999
47	*Dicranum himalayanum* Mitt.	N	*Moss Flora of China*. 1: 175. 1999
48	**Dicranum homannii* Boeck	U	*Handbok i Skandinaviens Flora,*

			Andra Upplagen 314. 1832
49	*Dicranum howelli* Renauld *et* Cardot	DWF	*Revue bryologique* 15: 70. 1888
50	*Dicranum japonicum* Mitt.	DWF	*Moss Flora of China*. 1: 176. 1999
51	*Dicranum johnstonii* Mitt.	DWF	*Journal of the Linnean society, Botany* 22: 300. 1886
52	*Dicranum kashmirense* Broth.	N	*Moss Flora of China*. 1: 178. 1999
53	*Dicranum klauteri* Reimers	U	*Hedwigia* 70: 363. 1931
54	**Dicranum kwangtungense* (P.C. Chen) T. J. Kop.	U	*Bryobrothera* 1: 200. 1992
55	*Dicranum leiodontium* Cardot	N	*Moss Flora of China*. 1: 178. 1999
56	*Dicranum leioneuron* Kindb.	DWF	*Herzogia* 17: 179-197. 2004
57	*Dicranum leucobryoides* Besch. *ex* Müll. Hal.	U	*Genera Muscorum Frondosorum* 285. 1900
58	*Dicranum levieri* Müll. Hal.	U	*Genera Muscorum Frondosorum* 285. 1900
59	*Dicranum linzianum* C. Gao	N	*Acta phytotaxonomica Sinica* 17: 115. 1979
60	*Dicranum longicylindricum* C. Gao *et* T. Cao	U	*Bryobrothera* 1: 218. 1992
61	**Dicranum longipilum* Müll. Hal.	U	*Synopsis Muscorum Frondosorum omnium hucusque Cognitorum* 1: 411. 1848
62	**Dicranum longirostratum* (P. Beauv.) Brid.	U	*Muscologia Recentiorum Supplementum* 1: 228. 1806
63	*Dicranum lophoneuron* Müll. Hal.	U	*Synopsis Muscorum Frondosorum omnium hucusque Cognitorum* 2: 589. 1851
64	*Dicranum lorifolium* Mitt.	DWF	*Moss Flora of China*. 1: 180. 1999
65	**Dicranum macrogaster* Müll. Hal.	U	*Hedwigia* 39: 252. 1900
66	*Dicranum majus* Turner	DWF	*Herzogia* 17: 179-197. 2004
67	*Dicranum mayrii* Broth.	DWF	*Moss Flora of China*. 1: 183. 1999
68	*Dicranum montanum* Hedw.	N	*Herzogia* 17: 179-197. 2004
69	**Dicranum morenoi* Müll.Hal.	U	*Hedwigia* 36: 97. 1897
70	*Dicranum muehlenbeckii* Bruch. *et* Schimp.	DWF	*Herzogia* 17: 179-197. 2004
71	**Dicranum myosuroides* DC.	U	*Flore Française. Troisième Édition* 6: 222. 1815
72	*Dicranum nipponense* Besch.	N	*Moss Flora of China* 1: 185. 1999
73	**Dicranum nitidum* (Dozy *et* Molk.) Dozy *et* Molk.	N	*Plantae Junghuhnianae* 3: 330. 1854
74	**Dicranum novae-hollandiae* Turton	U	*A General System of Nature* 2: 1717. 1806
75	*Dicranum novaustrinum* Margad.	U	*Lindbergia* 1: 127. 1972

76	*Dicranum obliquatum* Mitt.	U	Journal of the proceedings of the Linnean society 7: 148. 1863
77	*Dicranum ontariense* W.L. Peterson	DWF	*Canadian journal of botany* 68: 867-909. 1990
78	*Dicranum orthophylloides* Dixon	U	*Notes from the royal botanic garden Edinburgh* 19: 280. 1938
79	*Dicranum orthophyllum* Broth.	U	*Symbolae Sinicae* 4: 27. 1929
80	**Dicranum otii* (Sakurai) Sakurai	U	*Journal of Japanese botany* 27: 157. 1952
81	**Dicranum pachyneuron* (Molendo) Kindb.	U	*European and N. American Bryineae (Mosses)* 2: 190. 1897
82	**Dicranum pacificum* Ignatova *et* Fedosov	U	*Arctoa* 17: 76. 2008
83	**Dicranum pallescens* (Besch.) Müll. Hal.	U	*Genera Muscorum Frondosorum* 262. 1900
84	*Dicranum pallidisetum* (J. W. Bailey) Ireland	N	*The bryologist* 68: 447. 1965
85	*Dicranum papillidens* Broth.	N	*Akademie der Wissenschaften in Wien, Sitzungsberichte, Mathematisch-naturwissenschaftliche Klasse, Abteilung* 1, 133: 561. 1924
86	**Dicranum perichaetiale* (P. Beauv.) Brid.	U	*Muscologia Recentiorum Supplementum* 1: 204. 1806
87	*Dicranum peruvianum* H. Rob.	DWF	*The bryologist* 70: 317. 1967
88	*Dicranum petrophylum* G. Negri	U	*Annali di botanica* 7: 162. 1908
89	**Dicranum pinetorum* Griff.	U	*Calcutta journal of natural History and miscellany of the arts and sciences in India* 2: 497. 1842
90	*Dicranum polysetum* Sw.	DWF	*Herzogia* 17: 179-197. 2004
91	*Dicranum psathyrum* Klazenga	DWF	*Journal of the Hattori botanical laboratory* 87: 118. 1999
92	**Dicranum pseudacutifolium* OtnyUova	U	*Arctoa, a journal of bryology* 16: 163. 2007
93	*Dicranum pseudofalcatum* Seppelt	U	*The bryologist* 83: 591. 1980
94	**Dicranum pseudojulaceum* (Müll. Hal.) Müll. Hal.	U	*Hedwigia* 39: 259. 1900
95	*Dicranum pseudoleucoloma* Müll. Hal.	U	*Linnaea* 43: 397. 1882
96	*Dicranum pseudorobustum* Müll. Hal. *ex* Geh.	U	*Revue bryologique* 4: 53. 1877
97	*Dicranum rectifolium* Müll. Hal.	U	*Nuovo giornale botanico Italiano,* new series 3: 98. 1896
98	*Dicranum rhabdocarpum* Sull.	N	*Moss Flora of Central America. Part 1. Sphagnaceae--*

			Calymperaceae. *Monogr. Syst. Bot. Missouri Bot. Gard.* 49. 242 pp.
99	**Dicranum richardsoni* Drumm.	U	*Musci Americani;* or, Specimens of the Mosses Collected in British North America 104. 1828
100	*Dicranum rodriguezii* Müll. Hal.	U	*Genera Muscorum Frondosorum* 285. 1900
101	*Dicranum savatieri* (Besch.) Schimp. *ex* Paris	U	*Index Bryologicus, editio secunda* 2: 57. 1904
102	**Dicranum saxatile* Lag., D. Garcia *et* Clemente	U	*Anales de ciencias naturales* 5: 178. 1802
103	*Dicranum schensianum* Müll. Hal.	U	*Nuovo giornale botanico Italiano*, new series 4: 249. 1897
104	*Dicranum scoparium* Hedw.	DWF, N	*Herzogia* 17: 179-197. 2004.
105	**Dicranum scopellifolium* Müll. Hal.	U	*Nuovo giornale botanico Italiano*, new series 5: 169. 1898
106	*Dicranum scottianum* Turner *ex* Scott, Robert	N	*The Moss Flora of Britain & Ireland* pp. 210.1978
107	**Dicranum scrabrophyllus* Müll.Hal.	U	*Hedwigia* 36 : 84-144. 1897
108	**Dicranum seligeri* Brid.	U	*Muscologia Recentiorum Supplementum* 4: 59. 1819
109	*Dicranum semperi* Hampe	U	*Genera Muscorum Frondosorum* 297. 1900
110	*Dicranum setifolium* Cardot	N	*Moss Flora of China* 1: 189. 1999
111	*Dicranum spadiceum* J.E. Zetterst	DWF	*Herzogia* 17: 179-197. 2004
112	*Dicranum speirophyllum* Mont.	U	*Annales des sciences naturelles; Botanique*, sér. 2, 20: 295. 1843
113	**Dicranum splachnoides* Brid.	U	*Journal für die Botanik* 1800: 295. 1801
114	*Dicranum spurium* Hedw.	DWF	*Herzogia* 17: 179-197. 2004
115	**Dicranum strictum* (Dicks.) Sm.	U	*Flora Britannica* 3: 1218. 1804
116	**Dicranum stygium* Brid.	U	*Muscologia Recentiorum Supplementum* 4: 64. 1819
117	**Dicranum sulphureo-flavus* Müll. Hal.	U	*Index Bryologicus Supplementum Primum* 98. 1900
118	*Dicranum sumichrastii* Duby	U	*Mémoires de la société de physique et d'histoire naturelle de Genève* 20: 353. 1870
119	*Dicranum symblepharoides* Cardot	U	*Bulletin de la société botanique de Genève*, Sér. 2 1: 121. 1909
120	*Dicranum syrrhopodontoides* Müll. Hal.	U	*Hedwigia* 36: 96. 1897

121	*Dicranum tauricum* Sapjegin	N	*Herzogia* 17: 179-197. 2004
122	*Dicranum thelinotum* Müll. Hal.	U	*Nuovo giornale botanico Italiano,* new series 3: 98. 1896
123	*Dicranum thraustophyllum* Müll. Hal.	U	*Genera Muscorum Frondosorum* 297. 1900
124	*Dicranum toninii* Müll. Hal.	U	*Hedwigia* 36: 97. 1897
125	**Dicranum torquatum* Mitt.	U	*Journal of the proceedings of the Linnean society* 4: 69. 1859
126	*Dicranum transsylvanicum* Luth	DWF	*Herzogia* 17: 179-197. 2004
127	**Dicranum truncatum* (Müll. Hal.) Müll. Hal.	U	*Synopsis Muscorum Frondosorum omnium hucusque Cognitorum* 1: 410. 1848
128	*Dicranum truncicola* Broth.	U	*Akademie der Wissenschaften in Wien, Sitzungsberichte, Mathematisch-naturwissenschaftliche Klasse, Abteilung* 1, 133: 561. 1924
129	*Dicranum tubulifolium* Ireland	DWF	*The bryologist* 87: 355. 1984
130	*Dicranum undulatum* Schrad. *ex* Brid.	DWF	*Herzogia* 17: 179-197. 2004
131	*Dicranum viride* (Sull. *et* Lesq.) Lindb.	N	*Herzogia* 17: 179-197. 2004

Figure 1-3

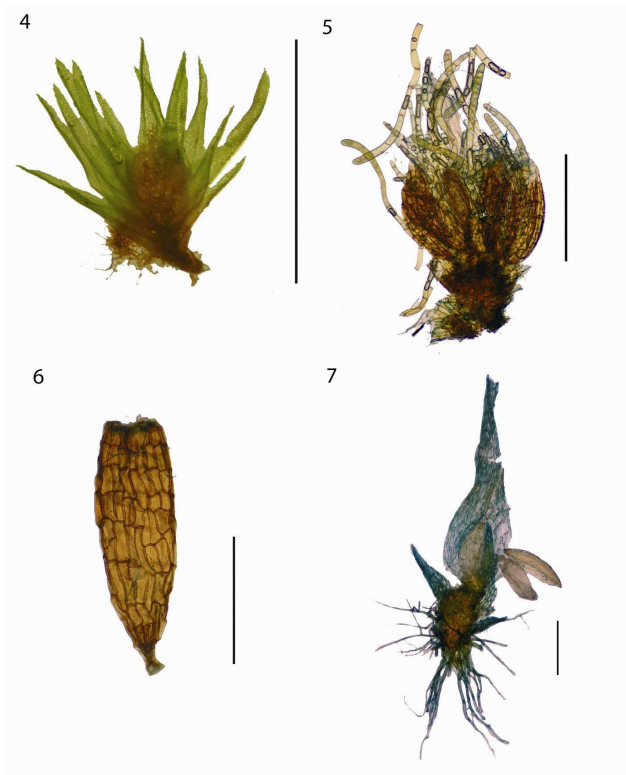

Figure 4-7

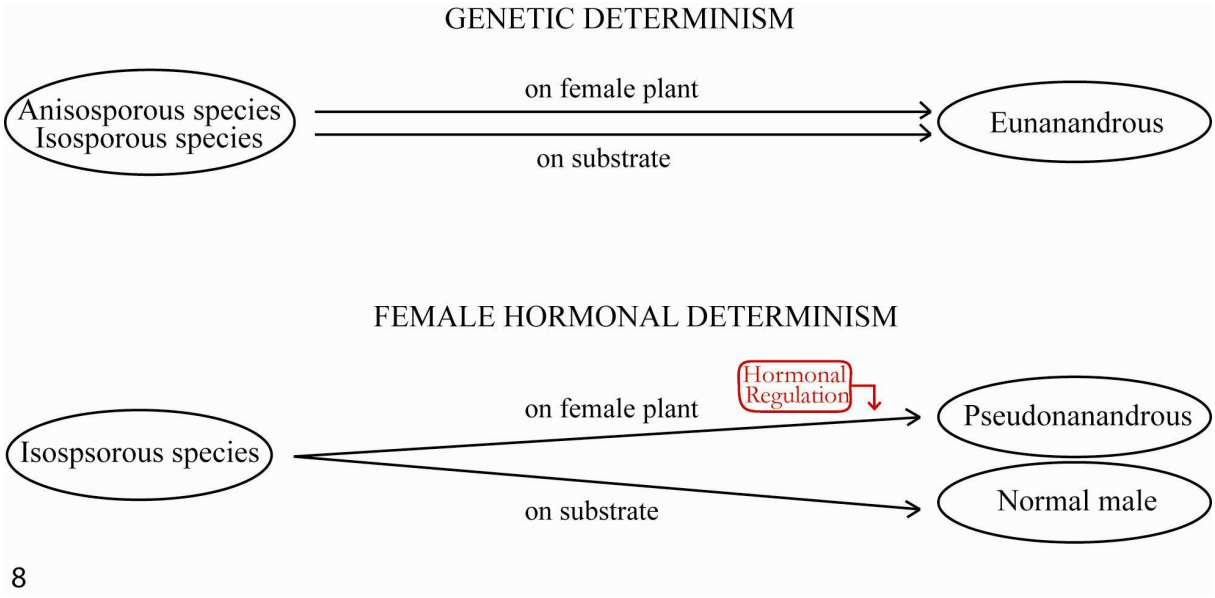
GENETIC DETERMINISM
Anisosporous species
Isosporous species
on female plant
on substrate
Eunanandrous
FEMALE HORMONAL DETERMINISM
Isospsorous species
on female plant
Hormonal Regulation
on substrate
Pseudonanandrous
Normal male
8

Figure 8

Article 5: Pichonet, A. & J. Bardat. 2011. *Campylopus joshii* (Leucobryaceae), a new species from Africa. Cryptogamie Bryologie. 32(1): 29-36.

Cryptogamie, Bryologie, 2011, 32 (1): 29-36

Campylopus joshii (Leucobryaceae), a new species from Africa

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(Received 5 October 2010, accepted 13 December 2010)

Abstract – *Campylopus joshii* Broth. *ex* Pichonet *et* Bardat is described as a new species from Uganda and the Democratic Republic of Congo. The type specimen was initially named *Dicranum joshii* Broth. *ex* Thér. and *Dicranum joshii* var. *latifolium* Thér. *et* Naveau but these names have never been validly published. The new species resembles the groups of *Campylopus* species with ventral hyalocysts and a ridged costa on the dorsal side but differs by the incomplete band of ventral stereids and single-celled dorsal lamellae.

***Bryophyta* / Dicranales / Dicranum / *Campylopus* / Uganda / Democratic Republic of Congo**

Résumé – *Dicranum joshii* Broth. *ex* Thér. et *Dicranum joshii* var. *latifolium* Thér. et Naveau sont des noms invalides car non publiés, cependant, ils représentent bien une nouvelle espèce de *Campylopus* pour l'Afrique (Uganda et République démocratique du Congo). Cette espèce est proche du groupe d'espèces à hyalocystes ventraux ainsi que du groupe à lamelles dorsales cependant il se différentie par une couche partielle de stéreides ventrales ainsi que par des lamelles unicellulaires.

***Bryophyta* / Dicranales / Dicranum / *Campylopus* / Uganda / République démocratique du Congo**

INTRODUCTION

Five species of the genus *Dicranum* Hedw. are currently recognised from Africa: *D. johnstonii* Mitt., *D. acanthoneurum* Müll. Hal., *D. borbonicum* Renauld *et* Cardot, *D. obliquatum* Mitt. and *D. petrophyllum* G. Negri (O'Shea, 2006). They are all considered as African endemics but are poorly known taxonomically and the latter three species are known only from the type collections. In addition, a sixth African taxon exists in *Dicranum*, *D. joshii* Broth. *ex* Thér. var. *latifolium* Thér. *et* Naveau, which was invalidly published. This paper deals with the latter taxon.

Dicranum joshii Broth. *ex* Thér var. *latifolium* Thér. *et* Naveau was based on a species recognised by V. F. Brotherus as "*Campylopus joshii*" but this is only

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a herbarium name which has never been published. Thériot and Naveau (in Naveau, 1927) transferred *Campylopus joshii* to *Dicranum* and described a new variety within it, but failed to provide the description of the species itself. According to the International Code of Botanical Nomenclature (McNeill *et al.*, 2006) both the species and varietal names are invalid.

Examination of the original material of *Campylopus joshii* and *Dicranum joshii* var. *latifolium* showed that this taxon actually represents an undescribed species of the genus *Campylopus*. The new species is currently known from two localities in Uganda and Democratic Republic of Congo (Fig. 1).

Fig. 1. Distribution of *Campylopus joshii* Broth. *ex* Pichonet *et* Bardat.

DESCRIPTION

***Campylopus joshii* Broth. *ex* Pichonet *et* Bardat, sp. nov.** Figs 2-13

Dicranum joshii Broth. *ex* Thér. *in* Naveau, *Bull. Soc. Bot. Belgique* 60(1): 21 (1927), *nom. inval. sin. descr. lat.* [Art. 32.1(c)].

Campylopus joshii Broth. *ex* Thér. *in* Naveau, *Bull. Soc. Bot. Belgique* 60(1): 21 (1927), *nom. inval. in synon.* [Art. 34.1(c)].

Dicranum joshii Broth. *ex* Thér. var. *latifolium* Thériot *et* Naveau *in* Naveau, *Bull. Soc. Bot. Belgique* 60(1): 21, *f.* 6 (1927), *nom. inval. sin. prior descr. spec.* [Art. 34.1(d)].

Diagnosis: *Caulis simplex vel parce ramosus, erectus, 8 cm altus, dense tomentus. Folia erecta, oblongo-lanceolata, sensim et longe acuminata, 9 mm longa. In transversali sectione foliae nervus compositus: una lamella magnarum ventralium rectangularium cellularum, una lamella in centrali parte magnarum globulosarum cellularum praetexta cum ventralibus et dorsalibus stereidis, dorsali superficia cum semi circularibus cristis ornata. Caetera ignota.*

Type: AFRICA. UGANDA: Entebbe Jashi, 2900 ft, coll. *P. G. Joshi* (1905), Hb E. Levier n° 6495 as *Campylopus joshii* Broth. (Holotype: H-Brotherus 0935037; isotype: PC 0128799).

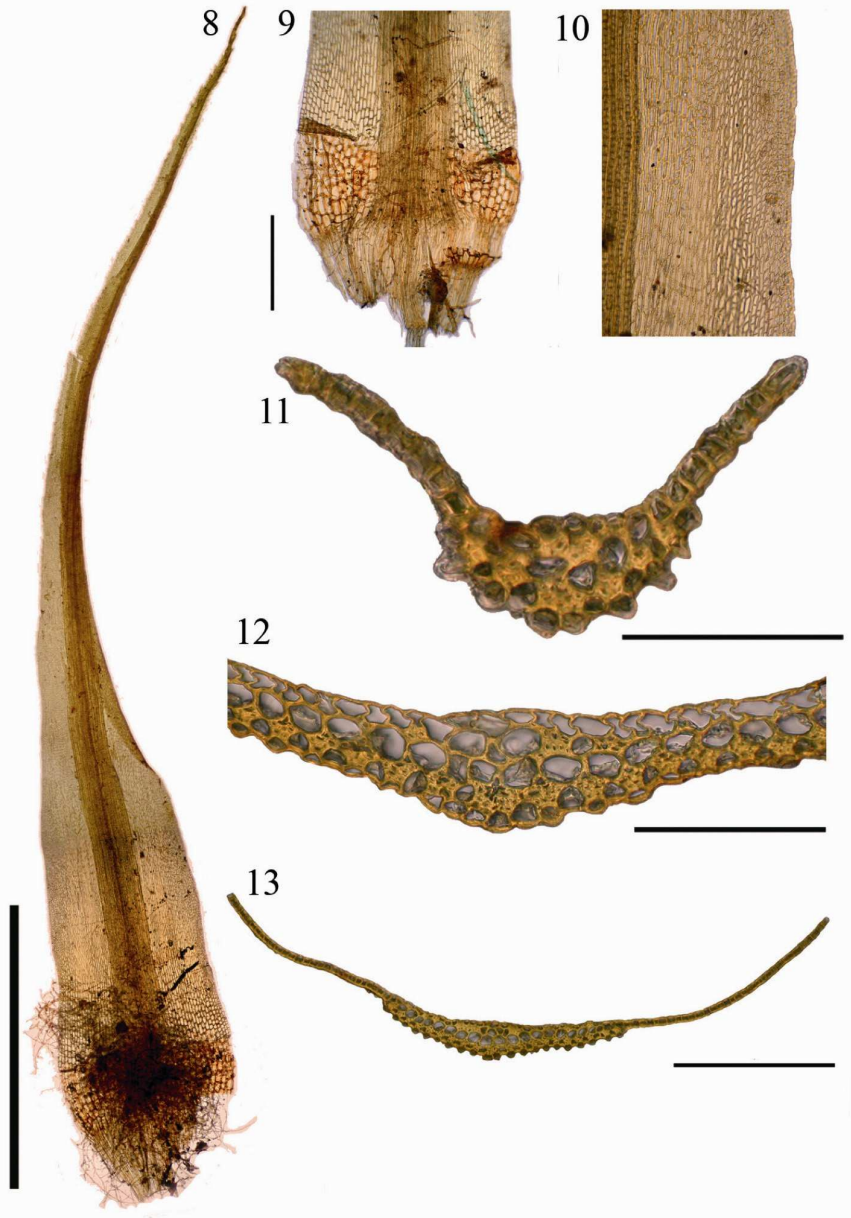
Paratype: AFRICA. DEMOCRATIC REPUBLIC OF CONGO: Angi (7 kil. à l'ouest de Rutshuru), 21 September 1914, coll. *Becquaert n° 5800* (PC 0128798 as *Dicranum joshii* var. *latifolium*).

Description: Plants robust. Stem erect, to 8 cm tall, densely tomentose. Leaves erect to slightly flexuose when dry, ovate-lanceolate or lanceolate, 9 mm long, 0.85-0.98 mm wide at base; margin serrulate or denticulate from upper third toward apex; costa 300-360 µm wide at base, filling most of subula and excurrent, without hair-point, denticulate dorsally in distal part, ridged at back with single-celled lamellae below, in transverse section with an incomplete ventral stereid band and a complete dorsal stereid band, one row of guide cells, a second row of adaxial guide cells in the middle of costa, one row of adaxial cells as large as guide cells at leaf base, cells thin-walled; alar cells extending to the costa, with reddish walls, forming prominent auricles; laminal cells not pitted, basal juxtacostal cells longly rectangular, 46.2 × 13.0 µm, becoming rhomboidal to quadrate towards the margins, 16.2 × 10.5 µm; upper laminal cells rhomboidal to short-rectangular; rhizoids smooth. Gametoecia and sporophyte not observed.

DISCUSSION

Campylopus joshii is known from two specimens. The holotype (H) is composed of about 15 shoots, while the isotype in PC is only one single shoot. The original material of *Dicranum joshii* var. *latifolium* (Figs 8-13), here treated as paratype of *Campylopus joshii*, consists of about ten shoots. The leaves and costa in the isotype of *C. joshii* are narrower than in the holotype (respectively, 624 µm

Figs 2-7. *Campylopus joshii*. **2.** Habit (scale: 1 cm). **3.** Leaf (scale: 500 µm). **4.** Leaf apex (scale: 250 µm). **5.** Transverse section of upper portion of leaf (scale: 100 µm). **6.** Transverse section of lower portion of leaf (scale: 100 µm). **7.** Transverse section of alar cells (scale: 100 µm). (All from the holotype, H-Brotherus).



Figs 8-13. *Campylopus joshii*. **8.** Leaf (scale: 2.0 mm). **9.** Leaf base (scale: 0.5 µm). **10.** Lower cells. **11.** Transverse section of upper portion of leaf (scale: 100 µm). **12.** Costa in transverse section in lower portion of leaf (scale: 100 µm). **13.** Transverse section of lower portion of leaf (scale: 250 µm). (All from *Naveau 5800*, paratype, PC 0128799).

vs 852 µm and 184 µm *vs* 304 µm) and if Thériot and Naveau (in Naveau, 1927) studied only the isotype, this may explain why they recognized the paratype as a separate "variety". Thériot and Naveau also described the sporophyte and the spores of *Dicranum joshii* var. *latifolium* but the original material lacks fertile plants (see Table 1). It is unclear whether there was only a single sporophyte available in the original material or whether there exists another fertile sample which could not be located.

Table 1. Morphological characters of the genera *Dicranum*, *Campylopus* and *Dicranodontium*, compiled from Frahm (1997), Allen & Ireland (2002), Allen (1989) and Limpricht (1890) – and of *Campylopus joshii* Broth. *ex* Pichonet *et* Bardat. Sporophyte characters of *C. joshii* after Thériot *et* Naveau (*in* Naveau, 1927).

	Campylopus joshii	*Dicranum* Hedw.	*Campylopus* Brid.	*Dicranodontium* Bruch *et* Schimp.
Costa	1/3 of the leaf base, ribbed and serrulate at the back, well developed dorsal stereid band, incomplete band of ventral steroids, ventral layer of hyalocysts present	< 1/3 of the leaf base, often ridged and serrate at back, with two stereid bands, ventral layer of hyalocysts lacking	1/3 to 7/8 of the leaf base, more or less ribbed and serrulate at the back, well developed dorsal stereid band, ventral layer of hyalocysts present	1/3 to 1/2 of the leaf base, more or less rough at the back above, with two stereid bands, ventral layer of hyalocysts lacking
Alar cells	inflated, reddish	often inflated, usually yellow-brown near the margin and hyaline toward the costa	usually clearly inflated, hyaline to brown to reddish	more or less inflated, hyaline or brownish
Seta	"flexuose" when moist	erect when moist	cygneous when moist, sometimes erect-flexuose	strongly curved to geniculate or cygneous when moist, erect-sinuose when dry
Capsule	horizontal, obloid, smooth when dry	erect, inclined or horizontal, elongate to cylindrical, more or less furrowed when dry	erect or curved, ovoid to ellipsoid, smooth to deeply furrowed when dry	erect, obloid-cylindrical, smooth to weakly furrowed when dry
Operculum	unknown	longly rostrate	rostrate	very longly rostrate (as long or almost as long as the capsule)
Stomata	unknown	present	absent	absent
Peristome teeth	bifid to 1/3-1/2	bifid to 1/3-1/2	bifid to 1/2	bifid to near the base
Calyptra	unknown	surface smooth, base entire	surface smooth base usually fringed by single-celled hairs	surface entire or rarely ciliate, base entire
Rhizoids	unknown	always arising from the stem or at the base of branches, never from the costa	frequently arising from the dorsal surface of the costa	on both surface of the costa, along stems and branch bases

The placement of a species in the genus *Dicranum* or one of its close relatives is sometimes difficult, especially when the specimens are sterile. For example, *Dicranum subporodictyon* (Broth.) C. Gao *et* T. Cao is included in *Dicranodontium* Bruch *et* Schimp., *Campylopus* Brid. or *Dicranum* by different authors. However, by its morphology the new species as described by Thériot and Naveau (in Naveau, 1927) and observed by the authors clearly belongs in the genus *Campylopus*, and not in *Dicranum* or *Dicranodontium* (Table 1). The costa is broad and occupies one third the width of the leaf base, with ventral hyalocysts, a partial band of ventral stereids and a well-developed band of dorsal stereids, and is serrulate at the back. The capsule is curved and oblong and the peristome teeth are divided to about 1/3-1/2 of their length. However, by its double row of guide cells in the middle of the costa in the lower part of the leaf the new species does not fit any of the *Campylopus* species known from Africa and Central America (Allen, 1989; Frahm, 1985, 1990, 1994; Frahm & Stech, 2006).

Campylopus joshii is closely related to the group of species which have a ridged costa on the dorsal side from the base to the upper part of the leaf, including *C. introflexus* (Hedw.) Brid., *C. pilifer* Brid., *C. aureonitens* (Müll. Hal.) A. Jaeger, but differs in having single-celled dorsal lamellae. The new species also resemble the group of species with a row of large hyaline adaxial cells (*C. pseudo-bicolor* Müll. Hal. *ex* Renauld *et* Cardot and *C. robillardei* Besch.) but differs from these by the presence of a dorsal and a partial ventral stereid band in the costa.

Acknowledgements. Our thanks are due to Amandine Allard for assistance in checking and digitalizing the *Dicranum* types in PC, Sanna Laaka-Lindberg for looking at the bibliography and type specimens, Professor Alain Couté for latinisation of the diagnosis, Professor John McNeill for assistance in the nomenclatural matters. We are also grateful to Professor S. Robbert Gradstein, Professor Jan-Peter Frahm and Professor Halina Bednarek-Ochyra for their critical comments on the manuscript.

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Conclusions et perspectives

Conclusions

Du questionnement originel ayant motivé ce travail de thèse (l'évolution des systèmes de reproduction chez les bryophytes et ses conséquences sur la diversité des espèces à travers l'étude du genre *Dicranum*), il est apparu très tôt la nécessité d'un cadre phylogénétique robuste support pour des inférences évolutives sur des traits de vie. De ce constat, une nouvelle problématique s'est déssinée cherchant à (1) connaître les relations de parentée entre les espèces du genre Dicranum, après (2) en avoir délimité les contours mais également à (3) estimer la variabilité moleculaire et morphologique de ses espèces à large répartition.

La première phylogénie moléculaire qui en résulte est riche de 26 espèces de *Dicranum* s.s.. Elle a permi un avancement des connaissances tant sur le plan technique que théorique. En effet, de nouveaux marqueurs moléculaires pour les Dicranaceae ont été testés et amplifiés avec succès (GapC, rpl32-trnL). Ce travail a églament augmenté le nombre d'assessions dans les banques de données moléculaires tant en nombre qu'en diversité spécifique.

D'un point de vue taxinomique, l'étude de trente-sept espèces de *Dicranum* s.l. a permi de valider le concept morphologique de onze d'entre-elles. Six autres taxa sont apparu polyphylétique. Plus surprenant, trois espèces cryptiques ont été mises en évidence pour l'Asie. La circonscription des espèces polyphylétiques et la délimitation des espèces cryptiques nécessitent une étude appronfondie des caractères morphologiques les discriminants et de leur pertinence vis-à-vis de l'origine de leur variation (génétique ou environnementale) comme cela a été illustré par l'étude de *D. majus* (Chap. 2). Il en resort également que le manque de signal moléculaire des marqueurs utilisés ne permet pas de résoudre la monophylie du genre *Dicranum* s.s.

Par ailleurs, l'importance de l'étude des spécimens types a été illustré plusieurs fois au cours de ces travaux. Elle a permis, par exemple, de rattacher au genre *Campylopus* l'espèce *D. joshii* ou bien encore de mettre en synonymie *D. johnstonii* avec D. scoparium, *D. mayrii* avec *D. montanum* (in prep.). Ceci a aboutit à une liste actualisée des espèces appartenant au genre *Dicranum*. Cette dernière fait état des connaissances à la fin de cette thèse et sera sans aucun doute ammenée à changer notamment à l'issu de la révision des 25 % des espèces connues uniquement à travers leur holotype.

Perspectives

A- Taxinomie

Les rares connaissances sur l'écologie et la biologie des espèces du genre *Dicranum* ainsi que l'absence de données taxinomiques actualisées pour un quart d'entre elles, rendent indispensables une révision morphologique et moléculaire rigoureuse du genre. Pour ce faire, il sera nécessaire (1) de réviser de façon systématique tous les spécimens types appartenant au groupe, (2) d'augmenter l'échantillonnage tant intragénérique qu'intraspécifique afin qu'il soit représentatif de l'aire de distribution de chaque taxon, (3) de rechercher et de développer des marqueurs moléculaires suffisamment informatifs au niveau intragénérique.

Dans cette perspective, 750 spécimens de *Dicranum*, récoltés dans la région du Tibet par Jürgen Kluge seront identifiés prochainement. De même, tous les spécimens type du groupe *Pseudochorisodontium* seront réexaminés.

Par ailleurs, l'incorporation de deux espèces endémiques d'Hawaï et de l'île de la Réunion, respectivement, *Dicranum spirophyllum* et *D. borbonicum* dans l'arbre phylogénétique du genre, combiné avec des fossiles préservés dans l'ambre (voir Frahm, 2000, 2004) permettraient de calibrer notre arbre phylogénétique. Nous pourrions ainsi disposer d'une estimation de l'époque à laquelle les spéciations intragénériques et intra sous-familiales se sont produites.

B- Processus évolutifs, mécanismes génétiques et conséquences populationnelles de certains caractères reproductifs

Deux caractères reproductifs notables existent au sein du genre *Dicranum*. D'une part, la présence d'organes plus ou moins spécialisés dans la reproduction végétative (production de gemmules, bris de feuilles) ; et d'autre part, l'existence de pieds mâles nains. On remarquera que ces caractères ne sont pas systématiques et qu'ils ne cœxistent jamais au sein d'une même espèce. Plusieurs questions sur les processus évolutifs, les origines et les mécanismes génétiques sous jacents, ainsi que les conséquences populationnelles, se posent alors. Ces interrogations se posent à la fois pour le genre mais également, plus largement, pour l'ensemble des bryophytes.

Les processus évolutifs :

La présence de structures spécialisées dans la **reproduction végétative** est-elle un phénomène rare ou commun chez les bryophytes ? Les espèces réalisant fréquemment de la reproduction asexuée présentent-elles parallèlement, une reproduction sexuée également fréquente ? Existe-t-il une corrélation entre l'écologie des espèces et la présence de structures de multiplication végétative ? Existe-t-il une corrélation entre la distribution des espèces et la présence de structures de multiplication végétative ? La reproduction végétative est-elle réalisée tout au long de la vie de la plante ou existe-t-il des phases de reproduction asexuée, de reproduction sexuée et de stérilité ?

Quand et combien de fois le **nanisme chez les mâles** est-il apparu chez les bryophytes ? Quelles sont les conditions ayant permis l'apparition et la maintenance du nanisme mâle chez les espèces de bryophytes dioïques ? Le nanisme mâle est-il un caractère ancestral ou dérivé ? Existe-t-il une corrélation entre l'écologie des espèces et le nanisme mâle ? Les mâles sont-ils plus petits ou les femelles plus grandes ? Les femelles des genres dans lesquels il existe du nanisme mâle sont-elle plus grandes que dans les autres genres comme cela existe chez les araignées du genre *Nephila* (Vollrath, 1998) ?

Biologie des populations :

Au sein d'une population, la **reproduction asexuée** apporte-elle à plus ou mois de variabilité génétique que la reproduction sexuée ? Quel peut être la taille d'un genet dans une population réalisant de la multiplication végétative ? Le flux de gènes entre populations physiquement distinctes est-il plus grand quand il y a de la reproduction végétative ou de la reproduction sexuée ?

Le **nanisme mâle** augmente-il de façon significative le succès reproducteurs des espèces dioïques ? Si oui, pourquoi ce caractère n'est-il présent que chez seulement 32 familles de bryophytes ? Quelle est la proportion d'inbreeding par rapport à l'outbreeding chez ces organismes haploïdes avec des mâles nains ? Existe-il une préférence maternelle pour les sporophytes les plus hétérozygotes, suggérant un évitement actif de l'inbreeding, comme l'a observé Szövenyi *& al.* (2009) ?

Génomique :

Comment se mettent en place les **zones de fragilité foliaires** permettant les bris de feuilles ? Quand ces zones de fragilités apparaissent-elles au cours du développement du gamétophyte ? Sont-elles présentes tout au long de la vie du gamétophyte ?

Quels gènes et quels composés chimiques sont responsables du développement des **mâles nains** chez les mousses ? Les mâles nains sont-ils des plantes néoténiques ?

Afin de répondre à ces questions, il faudrait (1) obtenir un arbre phylogénétique robuste qui servirait de base pour l'explication de processus évolutifs tels que la reproduction végétative et le nanisme mâle chez les bryophytes, (2) analyser les chromosomes sexuels afin d'étudier la démographie, la paternité ainsi que les pressions de consanguinité, (3) chercher des gènes et des composés chimiques qui pourraient être responsables des fragilités foliaires ou bien de la production de mâles nains.

C- De l'utilisation des microsatellites

Dans le cadre de deux stages de master, une étude de la diversité génétique de *Dicranum viride*, protégée en Europe (Natura 2000, Annexe 2 Convension de Bern) et *D. scoparium* a été menée sur des populations françaises, espagnoles et allemandes (Pichonet 2006, 2007). Cependant, la faible variabilité intraspécifique des marqueurs séléctionnés (ITS1, *rpl*32-*trn*L et *rbc*L) n'a pas permis de mettre en évidence de structuration géographique et/ou génétique

Dans le cadre de ce travail de thèse, il semblait donc nécessaire d'utiliser une méthode d'analyse plus résolutive en utilisant analyse des microsatellites (répétitions en tandem d'un motif de deux à dix nucléotides, encadrées par des séquences uniques ; et variable suivant les individus).

Cette étude préliminaire a été élargie à quatre taxon morphologiquement proches mais possédant des fréquences de reproduction sexuée et asexuées différents (*D. viride* : RS rare, RAs fréquente, *D. tauricum* : RS peu fréquente, RAs fréquente, *D. fulvum* : RS fréquente, RAs rare et *D. montanum* : RS rare, RAs fréquente). La zone d'étude inclut maintenant la France et la Chine.

L'objet de ce travail était de répondre aux questions suivantes :

(1) Comment définir génétiquement une population de bryophytes : est-ce à l'échelle d'une colonie ? Est-ce à l'échelle d'une parcelle forestière ? Est-ce à l'échelle d'une forêt ?

(2) Quelle est la structuration génétique au sein des différentes populations ?

(3) Existe-t-il des flux de gènes entre ces populations ?

(4) Les espèces clonales se caractérisent-elles par une plus faible diversité génétique ?

Les relations phylogénétiques entre ces quatre espèces n'étant basées que sur des caractères morphologiques, et compte tenu de l'aspect « espèce-spécifique » des microsatellites, quatre banques enrichies en microsatellites ont été développées selon la méthode de Billotte & *al.* (1999). Malheureusement, la mise au point n'a pu être finalisée.

En moyenne, dix marqueurs microsatellites ont été sélectionnés pour chaque espèce (**Figure 11**) et des tests de polymorphisme ont être réalisés.

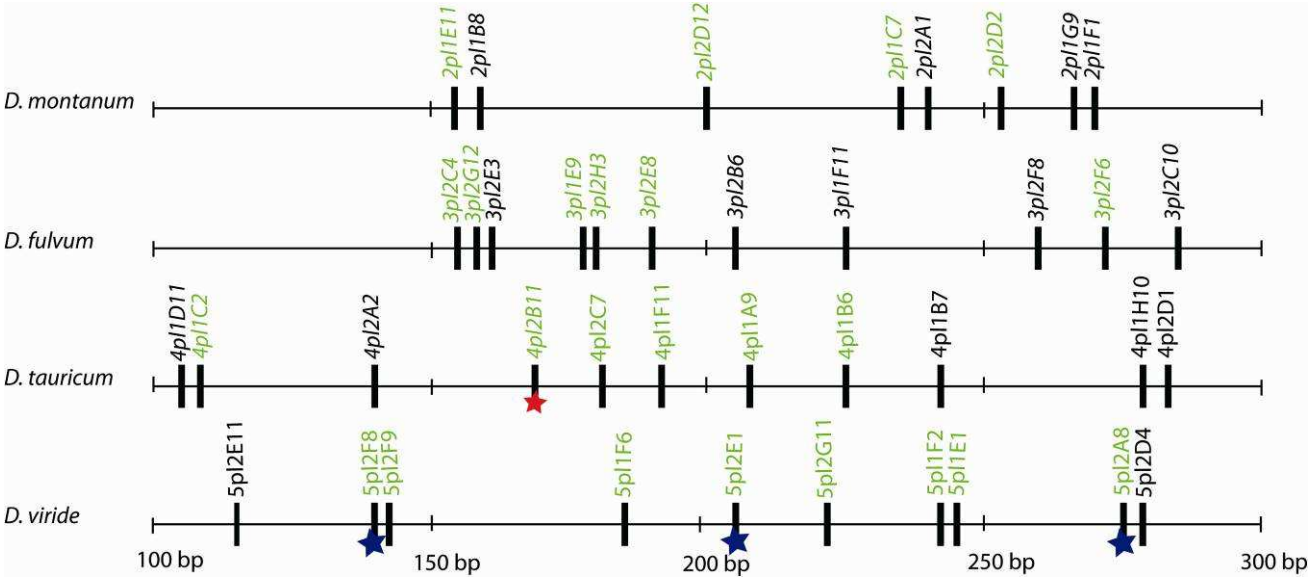

Figure 11 : Bilan des microsatellites sélectionnés, (vert) protocole d'amplification mis au point, (noir) protocole d'amplification à améliorer, (étoile rouge) cross-amplification réussie avec *D. montanum*, *D. fulvum* et *D. viride*, (étoile bleue) cross-amplification réussie avec *D. tauricum*

Cependant, les résultats taxinomiques mettant en évidence l'existence d'un patron géographique ségrégant les populations européano-américaines des populations asiatiques (cf. Article 2) nous obligent à repositionner notre étude en incluant les populations nord américaines. Ces résultats montrent également la nécessité de positionner les études populationnelles dans un cadre phylogénétique robuste afin d'éviter des mauvaises interprétations liées, par exemple, à l'existence d'espèces cryptiques (ex. *D. montanum*).

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